Vol. 34, No. 2 May 1947

# THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON
AGENTS FOR U.S.A.
THE UNIVERSITY OF CHICAGO PRESS
CANADA AND INDIA: MACMILLAN

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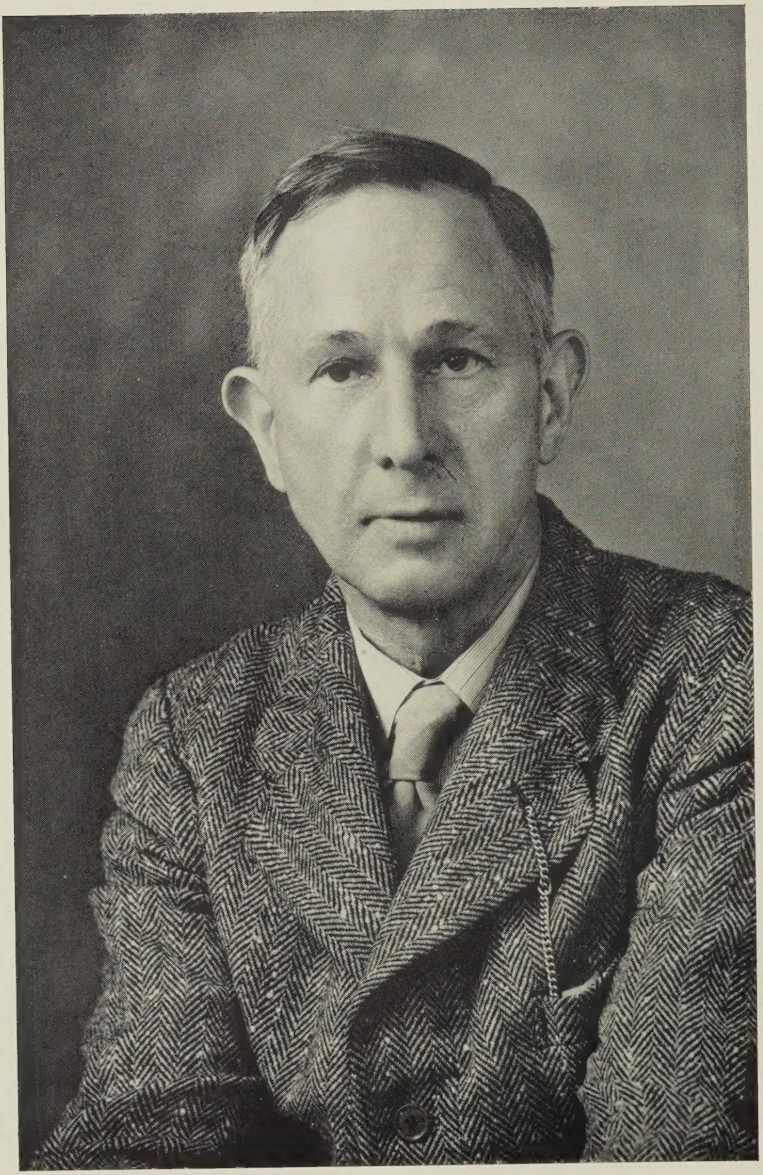

Dr C. B. WILLIAMS

*President of the Association of Applied Biologists*, 1944–1946

# THE FIELD OF RESEARCH IN PREVENTIVE ENTOMOLOGY

BY C. B. WILLIAMS, Sc.D.
*Rothamsted Experimental Station, Harpenden*

ADDRESS OF THE PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS, DELIVERED TO THE ANNUAL MEETING ON FRIDAY, 21 FEBRUARY 1947

As retiring President of the Association of Applied Biologists, I have to-day the mixed duty, privilege and pleasure of speaking to you on a branch of Applied Entomology which has always been of special interest to me, the anticipation and prevention of outbreaks of insect pests.

With the quite unprecedented success of direct-control methods by the new organic insecticides in recent years, one might almost begin to wonder if there will soon be any insects left alive to 'prevent', but for the moment I am not convinced, and have a feeling that the workers on indirect methods may come into their own again, perhaps even with more problems than before.

I have called my subject 'Preventive Entomology' for want of a better word, but it includes what some call 'Population Studies' and others 'Epidemiology'. I look upon it as a branch of Applied Ecology.

It is obvious that the actual prevention of an outbreak, although the primary object of the work, is likely to be a late stage in any investigation. First we must understand the causes of each outbreak; secondly, we must see if our understanding enables us to anticipate or account for particular outbreaks; and lastly, if it is possible so to alter the environment biologically or agriculturally that the threatened outbreak does not develop.

I propose to give, first, a brief introduction; secondly, an outline of the fundamental causes of the exceptionally great changes in insect numbers; and thirdly, a discussion of the more immediate causes of outbreaks, so far as they can be traced in the biological and physical environment.

An insect pest is any insect in the wrong place—from a human point of view; just as a rose bush is a weed when it is growing in a cabbage patch. Thus an insect may be a pest under one set of conditions, and not so under others. For example, the Painted Lady Butterfly (*Pyrameis cardui*) in this country is merely a 'thing of beauty', in France it is a pest of artichokes, while in North America it feeds almost entirely on thistles, and in its occasional years of abundance many suggestions have been made that it should be permanently established to control this troublesome weed. Unfortunately the Painted Lady does not take kindly to 'permanent establishment'; it is by nature a wanderer.

So almost any species of insect is a potential pest, and as over half a million different species are already known, and the probable total of existing species is far more than twice that number, the outlook is rather overwhelming.

However, no one, except in very special circumstances, objects to the presence of a small number of insects. Great damage is usually associated with the peaks of insect abundance, and the study of insect damage is therefore the study of changes in insect numbers both in time and space.

A problem in numbers cannot be separated from mathematical conceptions, and the first necessity in such a study is to introduce a reasonably accurate system of measurement for the factors on which the work is based. We have to get away as much as possible from vague statements that cannot be understood by others. What is the good of measuring temperatures to a tenth of a degree, and then studying their effects on insects in the field in which your only measure of abundance is 'rather common', 'not so common', 'more than a week ago', and so on. All such loose expressions should be given up unless some critical definition is given for each phrase for the particular problem in hand.

Once in East Africa I saw what I should have called a 'really gigantic' swarm of locusts—but it was also the first I had ever seen. Would a man who had seen many swarms think that this one was large or small? So I made a few rough calculations of the length, breadth, height, and density of the swarm, and came to the conclusion that it contained about twelve thousand million locusts. This is, of course, only an estimate, and subject to many errors, but at least it did not depend upon how many other swarms I had seen.

There is also another mathematical problem which must be mentioned before we proceed. In nearly all our work we try to infer general laws from a relatively small number of observations. We have in fact only a 'sample' of the facts of nature at our disposal, and this brings in some frequently overlooked difficulties.

In the first place it is most important, and not always easy, to see that our 'samples' are unbiased with regard to the problem we are studying. Let me give an example. For many years we have studied the migration of insects largely by collecting records of outstanding mass flights which have been recorded by naturalists and others in scientific or popular journals. But usually only if the flight was large enough to be conspicuous did it get recorded. We now find that frequently flights of insects are in two different directions at different times of the year, and that the flight in one direction may be in large numbers, and in the opposite direction in small numbers. Thus the evidence collected at random from periodicals through chance observations is biased in favour of the large migrations, while the smaller flights tend to be overlooked. Not until we had observers to watch continuously for large or small migrations alike did we get the unbiased evidence which has demonstrated the frequent existence of flights in two directions.

The second important point about sampling is that a good sample is by no means always a miniature reproduction of the thing sampled. The most perfect mathe-

matical sample frequently has properties which are characteristic of the sample and dependent on its size. For example, a small number of species selected at random from a large fauna or flora consisting of many genera will show a much smaller average number of species per genus than the original population. Again, if on sampling an animal or plant population we find that the number of species with one, two, and three, etc., individuals is arranged in that particular form known as a 'Poisson Series', then we do not set out to explain the existence of a Poisson Series, because this is only a result of sampling. What we would have to explain would be why all the species in the original population were *equally abundant*; because only when this occurs do we get a Poisson Series in the sample.

With these preliminary warnings that the mathematical approach to studies of changes in numbers of insect populations is necessary but full of pitfalls, I will turn to the questions, why are insects of such widespread importance to our crops and what are the fundamental causes predisposing them to this role?

I think that the reasons can be summarized briefly as due first to the enormous numbers of both species and individuals, and secondly to the very high birth-rate, and death-rate, and the relatively short life cycle.

With reference to the number of species, I have already estimated this as well over a million for the world as a whole, but it is also interesting to realize that in the British Isles there are already over twenty thousand species known, and that this is more than double all the other known species of living things put together—birds, beasts, reptiles and fishes, invertebrates, plants, fungi, etc. And yet for some curious reason the entomologist, who has in his field of research over half of the living creatures of the earth, is frequently accused of being narrow-minded.

When we come to the study of the numbers of individual insects we get figures which are almost astronomical. There are forty-five million human beings in Great Britain, and possibly about forty million pairs of nesting birds in England and Wales; but in a single acre of grass land or arable land, there may easily be over 100 million insects. Wireworms alone may reach over ten million to the acre. The total number of insects in the world is possibly about $10^{18}$ or a million, million, million!

But still more important from our point of view are the great changes in numbers which may occur in a relatively short time.

Politicians are worried because the human population of Great Britain shows signs of falling by about 5% in ten years. What would they say if it altered by 1000% in ten months. This may easily happen in an insect population. In the work done by H. F. Barnes on the number of larvae of wheat midges in 500 ears of wheat at Rothamsted, we have found numbers varying from 1000 to 33,500 over twenty years, and a change from 1100 to 28,200 has occurred from one generation to the next.

Pygmy Mangold Beetle (*Atomaria linearis*) was not noticeable on Barnfield at Rothamsted in 1932, but by 1935 there were six to seven million per acre and the crop was destroyed. And these two examples are insects which have only a single brood each year!

It is possible for a population to remain relatively stable only if the death-rate balances the birth-rate: both can be high or both low, but they must balance.

Now insects frequently lay 100 eggs, and sometimes 1000 or more; they usually have a life cycle in one year or less, and often there are several generations in a year.

If a pair of insects produces 100 eggs, then to have a steady population ninety-eight of these must die before they reach the stage of egg-laying. If, owing to very slightly more favourable conditions, only ninety-six die, then the size of the population will double in a single generation; if, on the contrary, ninety-nine die, then the population will be halved. Thus with a high birth-rate, such as is typical of insects, a very small change in the death-rate may produce very great changes in population. And if, as again is characteristic of insects, the generations are short, the fluctuations will occur more rapidly and more violently.

Thus we see that by the fundamental nature of their make-up, high birth-rate, high death-rate and short life cycle, the insects are subject to great fluctuations in numbers, and so become in special circumstances what we call 'pests' and liable to 'outbreaks'. Our difficulties are further complicated by the enormous number of different species—each with its own particular habits and habitat—and by the enormous number of individuals.

The next problem in our survey is the immediate causes which lead to any particular outbreak.

These are obviously any factor or factors which affect the birth-rate, the death-rate, or the length of life of the insect under consideration; to which must be added certain other factors which may lead to undue concentration of insects in one particular area.

These may be roughly classified as follows:

*Factors affecting the total population.*
  *The physical environment.*
    Geology and geography.
    Climate and weather.
  *The biological environment.*
    Food supply.
    Parasites and predators.
    Competition.
*Factors affecting the distribution of the populations.*
    Migration, or deliberate movement.
    Drift, or accidental movement by natural causes.
    Accidental distribution by human agency.

Some of these factors may vary from place to place at the same time, others may vary from time to time at the same place, producing respectively local or seasonal outbreaks. Some of them can be altered, or may even be dependent upon human activities. Others may be impossible to alter, but their effects can be minimized or emphasized by our interference.

The climate is one of the major factors in determining whether or not an insect can survive permanently in a locality. The weather—that is, all the fluctuations of the climate—determines very largely what numbers will exist at any particular moment. The study of the relation of weather conditions to insect activity and insect abundance is therefore one of the main fields for research in Preventive Entomology.

Much has been done in the last twenty-five years or so to understand the relation of insects to temperature, rainfall, humidity and other physical conditions. Progress has been made, particularly in analytical work on a single factor under controlled conditions in the laboratory. But this is not enough. Whatever may be the difficulties, the ecologist must go into the fields where the conditions are never simple, and try to understand what is happening under the complexity of natural conditions. If a sufficiently large number of observations can be made, and if, as I have emphasized before, these can be expressed in a numerical form, then it is possible to apply statistical methods, and at least get results which may be significant and capable of further testing against biological events in the field.

For example, by catching insects in a light-trap at Rothamsted for over 1400 consecutive nights we were able to show that the number of insects caught is doubled by a rise in minimum temperature of 5° F. From the average catch for forty-eight successive months, we were able to obtain formulae for estimating the population level in any one month from the rainfall and temperature of the three preceding months; and our calculated results were in good agreement with the observed facts.

Weather conditions can, of course, act directly on the particular insect pest under consideration or indirectly through its parasites, or its food supply—through its competitors, or by affecting its movements. The sorting out of these different cross-relations is one of the doubtful privileges of the field ecologist.

Turning from the influence of weather to that of food supply we may note that in general the complete failure of food supply seldom acts as an important control of insects in nature. Usually, before this happens, other factors such as parasites and disease, or changes of season, play an important part. On the other hand, both quantity and quality of the food supply can sometimes have a considerable effect by influencing the fertility of the insects.

The problem of food supply in insect pests is also much more easily under the control of the farmer. He can, within limits, plant different crops or more resistant varieties; plant at different seasons, or in a different rotation, and so make it more difficult for the insects to find food at the particular time that they require it most.

An example of this is the damage done to Meadow Foxtail by three Cecidomyid midges. Barnes has shown that the midges hatch out just at the time when the flowering heads of the grass are suitable for oviposition. It is not easy to alter the date of emergence of the midges, but, by cutting back the grass a month or so before flowering, the date of flowering can be delayed. As a result of this the midges hatch

out and die without encountering any proper seed heads in which to oviposit, and when the grass flowers a few weeks later than normal there are practically no midges and the damage is greatly reduced. The midge and the host plant are put out of tune in their seasonal cycle.

So we see that in this question of food supply, as well as in other aspects, the study of 'Phenology', or the variation of the dates on which things happen in the course of seasonal changes, is of importance to us. On an average an insect is in tune with its food plants, but unusual weather conditions, or the activities of the farmer, may put them out of step with often quite striking results

Much other work has been done on the relation of food supply to insect abundance, particularly in the Aphidae, and in this group we have the special problem of winter host-plants. Only recently a suggestion has been made to prevent the serious outbreaks of Black Aphis (*Aphis fabae*) in this country by destroying its winter hosts. This will be mentioned again later.

Next we come to the interrelation of host, parasite and predator—in its practical application commonly called 'Biological Control'. The object of our work here is to restore the fluctuating balance, already upset by man's interference, to a level below that of frequently recurring outbreaks.

There are two main methods of practical application, the introduction of new parasites, and the encouragement of those already present. The most striking successes have been achieved with introduced pests in countries of an 'Island' type with a relatively simple association of living creatures. I do not know of any great success in a continental area with a pest already native to that district.

The use of such methods in particular practical problems has been frequent, and the successes have justified the failures; but the fundamental principles have been little studied. The advantages or disadvantages of the simultaneous introduction of several parasites or predators is still a vexed question. Before the last war the Imperial Institute of Entomology was advertising for an entomologist with good training in mathematics to study the fundamental relations of host and parasite, but so far as I know the post was never filled. Now the Imperial Parasite Service has moved to Canada, and we are without any centre for the study of Biological Control either practically or theoretically. Conditions in England do not seem to be suitable for great success, but that is no reason why we should drop out of the field. Our Colonial Empire sometimes looks to us for a lead in research!

The problem of competition, which has been little studied until recently, is concerned chiefly with all those interrelationships between one insect species and another which cannot be included in the simple relation of host and parasite.

Every individual of every species is in competition with other individuals of its own species, and with those of other species, for food, space, shelter and many other possibly quite small necessities of daily life; and as a result no species of insect can alter its numbers without affecting the numbers of many others—perhaps all—with which it comes into contact.

So all the species of insects, of animals and of plants in any environment are in loose association, in which, however, there is some semblance of order.

We have recently shown at Rothamsted that in any random sample of the insects taken from a wild population there is an order in the relative abundance of the different species; and we think that we have found a mathematical law which fits in with the observed order. Be this as it may, the fact remains that there is an order in the samples, and so there must be an order in the populations from which the samples were taken. Any law or order or relative abundance must be fundamental to our study of changes of numbers, for if one species changes its position in the series then all the others must also change to produce a new balance.

The very abundance of a species brings its own dangers, spread of disease, shortage of food, ease of prey to its enemies, conspicuousness, and so on. Many environmental factors which tend to reduce numbers act increasingly strongly as the insect becomes more abundant and so force the population back towards the average, which is so frequently represented as relatively stable. There is indeed a balance, but it is that of an unskilled tight-rope walker who is constantly swaying from side to side to avoid a fall.

Low numbers also bring their difficulties, particularly that of finding a mate, and in this case the new factor acts to increase the departure from the average. Thus it is obvious that the fundamental study of the 'balance' of insect numbers must be included in any investigation of Preventive Entomology.

Lastly we come to those factors which do not necessarily affect the total numbers of an insect existing at one moment, but their distribution—resulting in concentration in dangerous numbers in special areas.

The first of these and possibly the most important, although little studied, is deliberate migration. It is of course well known and its economic importance recognized in locusts, but it occurs also in many other orders of insects. Several species of Lepidoptera are known to fly over a thousand miles in the course of their adult life, and the flight may be quite regular in season, direction and route, though usually very variable in numbers.

The problem of migration is of importance both in control and prevention, but more so in the latter, for the farmer can do little to prevent the rise to dangerous numbers of an insect, when the increase may be brought about by favourable conditions a thousand miles away. At present all he can do is to lay in a store of insecticides and be ready for the worst.

Migrant pests are those which specially need international co-operation, and it is gratifying to see that in an otherwise quarrelsome world, international co-operation on locust control is at a higher level than ever before, and we can pride ourselves on the fact that the central co-ordinating office for much of this work is in London.

As an example of the impact of migration on pest control I may mention the Greasy Cutworm (*Agrotis ypsilon*). This is in Egypt a pest of grain in winter, and

occasionally in spring a pest of seedling cotton. Much money was at one time spent on trapping the adult moths by means of a sugary bait, which was particularly successful in attracting great numbers in the spring. Further study showed, however, that the insect was a migrant, and spent the summer months breeding 'somewhere in Europe'. All the females caught in the spring were potential emigrants, and none had developed eggs in their ovaries. So that all we were doing by our trapping was spending money to prevent the moths leaving of their own accord, and at their own expense.

Another interesting example of how difficult it is to keep the different branches of our subject separate is the case of the migratory predacious wasp (*Sphex aegyptiacus*) that I found in East Africa. The wasp digs holes in the ground and puts in paralysed adult locusts as food for its own young. It has developed a habit of migrating along with the locust swarms on which it preys. I had lived nearly two years at Amani in Tanganyika before the first locust swarm arrived, and in that time I had not seen a single individual of the *Sphex*. The first locust swarm arrived about 11 a.m. one morning, and at midday in less than one hour I picked up 168 *Sphex* on about 25 square yards of path, all busy digging holes and dragging in locusts; and there were as many when I had finished as when I started. Next day between 1 and 2 p.m. the locusts began to depart, and by 2.15 there were only three *Sphex* to be seen on 100 yards of roadway where two hours previously there had been thousands. The *Sphex* had even abandoned holes half-finished or with the paralysed locusts lying alongside.

Still another remarkable case of a migratory predator (but this case on a comparatively sedentary host) is the Ladybird (*Hippodamia convergens*) in California. The adult beetles hibernate in small colonies of several thousand individuals all bunched together in the hills to which they migrate over a distance of a hundred miles or more in the autumn. For many years the beetles were collected in great quantities during the winter by entomologists and kept in a cold storage for use to control outbreaks of aphids during summer. The beetles were thawed out and liberated wherever they were wanted. But the method was found to be useless and gradually abandoned. Why? Because one of the fundamental principles of the migrating habit had been overlooked. When the insects naturally wakened from their long winter sleep their first and overpowering instinct was to fly the hundred miles or more necessary to take them back to their summer quarters. When artificially thawed out in an orchard, off they set on their long flight and were never seen again, at least in that district!

Each year adds to our list of migrant insects and to the information about known migrants, and there is little doubt that the old idea of insects being confined to a relatively small area must be seriously modified in many species. Movements of hundreds of miles are comparatively frequent, and movements of well over a thousand miles are not unknown.

Next comes the problem of the accidental distribution of insects by wind currents,

or more rarely by water. The aerial plankton consists, of course, chiefly of small winged insects, but also includes young spiders supported on their gossamer and even, as the entomologists in America have demonstrated, of small hairy caterpillars such as the just-hatched larvae of the gipsy moth. In a trap fixed to an aeroplane in U.S.A. a springtail (Collembola) was found at 11,000 feet!

Undoubtedly the most economically important of the airborne insects are the Aphidae, and hundreds of these were once found on the snow at Spitzbergen, at least 400 miles from their nearest food plant. Prof. Hardy, who has made a special study of aerial plankton, estimates that on a fine summer day there are several million insects, mostly Aphidae, over the North Sea in the area which is at least 50 miles from the nearest coast. There are occasional records of disasters occurring to such populations when hundreds of millions of Aphidae are washed up along our coast-line. Along with these there are at times numbers of Ladybird beetles and Syrphid flies, somewhat recalling the case of the *Sphex* and the locusts already mentioned.

This is not the place to go into details, but it is easy to see that drift on air currents is an important part of the 'insect outbreak' problem, and I am glad to say that we have recently had an addition to the entomological staff at Rothamsted to study it in more detail.

Finally, there is the important but artificial problem of the accidental transmission of insects by human agencies—ships, trains, motors and aeroplanes. The problem was already serious 50 years ago, as the long list of major pests accidentally introduced from Europe into North America shows; but to-day, with the trans-ocean crossings from continent to continent being accomplished daily, the chances of introduction are enormously increased. Already most stored-produce pests are found throughout the greater part of the world; and the problem of transmission of disease-carrying mosquitoes or yellow-fever infected patients is one of the most serious questions facing medical entomology.

Although not exactly a field for fundamental research, the whole question of the organization of inspection, customs and quarantine is an important part of preventive entomology.

Before summing up I should like to deal with one criticism of my point of view that is continually being put forward, namely, what is the use of this knowledge? Even if you know that a particular pest is encouraged by wet weather or by dry, what can the farmer do about it? He cannot alter the weather!

My answer is that from the very beginnings of agriculture in prehistoric times the farmer has spent all his time altering the physical and biological environment in which his crops grow. If he cannot alter the rainfall, he *can* alter the effects of the rain that falls. By drainage if necessary—by spacing—by choice of site—by choice of planting season—by shading—by deep or shallow cultivation—by rotation of crops—by choice of crop varieties; in all these and many other ways, the farmer is continually altering the environment to suit his needs. Some problems are easy,

some difficult, but there will always be progress and always some old or new difficulties to be overcome.

The study of pest and plant in relation to environment may also give an answer to one of the perennial questions that come to entomologists—when to use 'direct' insecticide control and when to use 'indirect methods'. The general answer is that if the best environment for the crop is the same as the best environment for the pest, direct methods are the only immediate hope. If, however, the optimum conditions for the crop and the pest are not the same, an improvement can always be brought about by changing the conditions away from what is best for the pest and towards what is best for the crop.

Although in many cases the two optima differ, the idea that a crop grown under the best possible conditions is bound to be free from pests is a fallacy. For millions of years each species of insect has been getting closer and closer in relation to its particular host plants, and it is only natural to expect that in some cases the relation may be so close as to leave little room for indirect methods of control or prevention.

To sum up: I anticipate in the future a kind of 'Plant Health Advisory and Research Service' which will do for plants and their pests what the Medical Public Health Service is doing to-day for human beings and their diseases.

You must recollect however that it is only fifty years ago since Ross was told that his business was to *cure* people and not to meddle with mosquitoes. And yet already to-day almost the major portion of the work of the medical world is to prevent disease occurring and spreading.

The research side of our organization—call it what you will—should at least cover the fields of ecology that I have outlined: (1) the relation of the insect to its physical environment and the changes brought about by agriculture, (2) the relation of the insect to its food supply, including continuity of food and the development of varieties resistant to attack, (3) the relation of insects to their parasites, predators and hyperparasites, and the problems of biological control, (4) the competition between associated species in a mixed ecological community, (5) the problems of migration and drift which are concerned with the concentration of insects into one locality, and (6) the somewhat artificial problem of transport of insects by human agency, both deliberate and accidental.

Lastly, I should like to emphasize once more that while analysis of single factors under controlled conditions in the laboratory is essential to progress, so it is equally important that work should be carried out in the field where every insect is under the influence of all the complex factors of the environment.

I have outlined a big programme of research which will occupy many men for many years. Much of the work is already being carried out by individual workers and small departments in all parts of the world, but I would like to see still more effort expended on the fundamental problems.

I have already in my own life seen great changes in entomology. In 1900, when I was about ten years old, one man was entomologist and botanist to the whole of

Canada; and I had left the University and done several years research before our own Ministry of Agriculture had a single full time Advisory Entomologist. So perhaps it is not asking too much, if expansion continues in the future as rapidly as it has done in the past, to hope that there will be men available to carry out this type of work, with facilities for field research, and with time to think.

The results of such work will however be slow to come and will never be spectacular, nor will much credit go to the investigators. It is easy to say 'What a beastly nuisance this cold of mine is. Why cannot doctors do something about it.' But, if doctors were at last able to abolish colds, how many people, and for how long, would say, 'How clever it is of the doctors that I have not got a cold.'

And so it will be with the entomologists.

# THE BEHAVIOUR OF ADULT CLICK BEETLES OF THE GENUS *AGRIOTES* (*A. OBSCURUS* L., *A. LINEATUS* L., AND *A. SPUTATOR* L.)

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(With 4 Text-figures)

Various methods of trapping click beetles were tried, the aim being to devise a method which could be used for population and movement studies in grass fields. It is shown that a trap consisting of a 3 in. thickness of hay over closely clipped grass is suitable for this purpose. Annual and daily activity are discussed. Maximum activity occurred in late May and early June, and during the afternoon and evening. The beetles showed a preference for the most humid sites during dry weather. Numbers fell as the hedge was approached and very few occurred under the hedge. Marked beetles were released, and the maximum distance at which they were recovered was 26 yards (23·8 m.). The majority moved in a very restricted area.

## Introduction

Most of the click beetles are retiring in their habits and, although they may be present in great numbers in a limited area, they remain hidden from view. Little is known, therefore, of their daily movements. Most of the references are collectors' notes giving lists of hiding places, such as under stones, at roots of grass, in moss, grassy places, etc.

Curtis (1883) speaking of Elaters says: 'They walk and run like dogs, with their heads and trunks declining, their noses close to the ground; when they leap, their legs are applied closely to their bodies, and by the same means they fall down when the plants are approached upon which they are resting.' Also: 'It is now become an active beetle, running up the grass, and readily flying to flowers, to which it resorts for food.' Although he does not mention the species referred to, his figure of *Agriotes lineatus* L. is 'represented flying'. Masaitis (1929) states that *A. sputator* L. is usually on the wing in the evening. Mesnil (1930), and later with Balachowsky (1935), speaking of the three chief pest species say: 'They do not seem to fly. We lived many years in an area where *Agriotes* were abundant without ever seeing the insects use their wings, not even in the day, evening or night.' Langenbuch (1932) never found adults of *A. obscurus* L. except on the ground. Subklew (1934) states that *A. obscurus* L. was seldom seen flying and *A. lineatus* L. never. Fryer (1941) records netting *A. obscurus* L., *A. lineatus* L. and *A. sputator* L. on the wing at one time or another, always between sunset and dark.

It was therefore felt desirable that the movements of the three main grassland species of the genus *Agriotes* should be studied. The aim was to discover their habits

from emergence in the soil to pairing and egg-laying. These might have a bearing on (1) the possible migration from field to field, hence indirectly on the repopulation of arable land put down to grass; (2) the possible control of the pest in the adult stage.

### Choice of fields for experiments

Field studies were made in 1944 and 1946; in both years the fields chosen had wireworm populations of over a million per acre determined by the hand-sorting method. In 1944 the field was on a hillside with southern aspect, on Keuper Marl; the turf was worn-out meadow, surrounded by hedges about 10 ft. high. The lower part of the field, an area of about 2 acres, was used for the investigations. A wireworm count on this part indicated a population of about 1,600,000 per acre. In 1946 a reseeded field on alluvial deposits of the river Soar was chosen. It is flooded for a few days in most years, and wireworm counts before and after reseeding indicated a population of about 1,100,000 per acre. These populations ensured that there would be large numbers of click beetles present during the summer.

### Trapping methods

*Sweeping.* Net sweeps gave very poor results. In 1944 ten 15 min. sweeps over a period of 18 days in the second half of May resulted in the collection of two *A. sputator* and four *A. obscurus* from the hedge bottoms, none from the hawthorn hedges and one *A. lineatus*, nine *A. obscurus* and four *A. sputator* from the field. In 1946 six sweeps were made of the field, the hawthorn hedge and the bottom of the ditch every day from 13 May to 4 July and yielded but two *A. obscurus* from the field.

*Sticky traps.* In 1944 ten 6 in. wooden squares coated with grease-banding substance and placed on the ground caught one *A. lineatus* and one *A. sputator* in the period 12–30 May. Ten similar traps placed vertically on posts at heights between 1 and 6 ft. caught one *A. lineatus* at 2 ft. from the ground in the period 12 May–7 July.

In 1946 three cylinders, 5 in. in diameter and 6 ft. high, were erected and coated with grease-banding substance. No beetles were caught on these over the period 13 May–4 July. In addition a screen was set up consisting of two sheets of muslin 8 ft. 6 in. high by 4 ft. 6 in. wide, set at right angles to each other, and coated on both sides with grease-banding substance. One *A. obscurus* was caught on this on the side facing south at a height of 5 in. from the ground.

Sticky traps to catch aphides were placed in different localities in the Midlands for a number of years (for description see Broadbent (1946)). In all, two specimens of *A. sputator* and one of *A. obscurus* were caught on these, all of them near the base of the trap at 3 ft. from the ground.

During 1946 attempts were made to induce the beetles to fly. They were placed on the tops of flowers and grasses, but most of them immediately ran down the stalk to the ground. Others ran over and under the flowers; if they reached the tip

of a narrow leaf they attempted to turn. If this was impossible they fell to the ground but never flighted.

*Stones.* Under stones is a regular habitat for collectors for a number of elaterids. This method of collection was tried in 1946 by placing large stones in the field, but with no result.

*Grass and hay traps.* In various forms these have been used by workers for many years, and heaps of clover, lucerne, etc. scattered about the field have been recommended as a measure of control for the adults.

In preliminary experiments in 1944 hay and fresh grass traps, each being 1 ft. square and about 3 in. deep, were tried on closely clipped and on unclipped meadow. The yields from ten traps of each type, from eight examinations over the 3 days 13–15 June, were:

| | |
|---|---|
| Fresh grass traps on clipped meadow | 71 |
| Fresh grass trapps on unclipped meadow | 67 |
| Hay traps on clipped meadow | 164 |
| Hay traps on unclipped meadow | 119 |

It was estimated that up to a third of the beetles was lost in lifting the traps from unclipped meadow. In general, *A. lineatus* tended to be left behind when the trap was lifted, *A. obscurus* and *A. sputator* remaining amongst the hay or grass.

In 1946 the following types of trap were operated:

(1) Hay on clipped grass.
(2) Hay on clipped grass surrounded by 6 in. of bare soil.
(3) Hay on bare soil.
(4) Grass on clipped grass.
(5) Grass on clipped grass surrounded by 6 in. of bare soil.
(6) Grass on bare soil.
(7) A pit, 4 in. deep, with clipped grass bottom and filled with hay.
(8) A pit, 4 in. deep, with bottom of bare soil and filled with hay.
(9) A pit, 4 in. deep, with clipped grass bottom and filled with grass.
(10) A pit, 4 in. deep, with bottom of bare soil and filled with grass.

All the traps were 1 ft. sq. with 3–4 in. depth of hay or grass.

Table 1 gives the average number of *Agriotes* spp. taken per trap per day during weekly periods in 1946. The traps were cleared each morning at the same time in the same order. Table 2 gives the average total yield per trap for the different species throughout the period 13 May–4 July 1946. It will be seen that the different species were caught in similar ratios in all types of trap, i.e.

*A. lineatus* 8·6%, *A. obscurus* 55·5%, *A. sputator* 35·9%.

In 1944 the ratios were:

*A. lineatus* 9·9%, *A. obscurus* 15·5%, *A. sputator* 74·6%.

It is interesting to note the different relative proportions of *A. obscurus* and *A. sputator* in the two fields, which were about 1 mile apart, but on different soils. Although

traps 1–5 were successful, it was felt from the data collected in both years from the different sites that the most efficient type with which studies of population might be carried out was type 1, hay on close-clipped turf. That this type of trap was dealing with a representative proportion of the whole population may be shown as follows:

In 1944 the peak average catch of *Agriotes* spp. per square foot trap was eleven, i.e. 479,160 per acre. The figures obtained by the wireworm survey teams in the Midland Province, using the hand-sorting method, have shown that a one-third reduction in numbers of wireworms normally takes place each year after turf is

TABLE 1. *Average number of* Agriotes *spp. trapped per day during weekly periods*, 1946

| | Trap type no. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 13–19 May | 0·1 | 0·2 | 0 | 0 | 0 | 0 | 0 | 0·1 | 0 | 0 |
| 20–26 May | 6·2 | 2·3 | 2·9 | 3·1 | 2·0 | 2·5 | 1·3 | 1·3 | 2·1 | 0·8 |
| 27 May–2 June | 8·1 | 8·5 | 7·1 | 5·8 | 4·6 | 3·4 | 3·1 | 2·5 | 4·5 | 1·7 |
| 3–9 June | 5·0 | 2·4 | 3·1 | 2·9 | 3·1 | 2·1 | 1·5 | 1·6 | 2·4 | 2·1 |
| 10–16 June | 2·0 | 0·8 | 1·5 | 1·3 | 1·3 | 0·7 | 0·1 | 0·4 | 0·4 | 0·4 |
| 17–23 June | 2·0 | 0·8 | 0·7 | 1·5 | 2·5 | 1·0 | 0·9 | 1·0 | 0·5 | 0·1 |
| 24–30 June | 1·5 | 0·2 | 0·4 | 2·3 | 3·4 | 1·1 | 0·6 | 1·1 | 1·4 | 0·8 |
| 1–4 July* | 1·3 | 2·1 | 1·2 | 1·0 | 1·4 | 2·1 | 1·1 | 1·0 | 1·9 | 0·1 |

* 4 days only.

TABLE 2. *Average total yield per trap for each species throughout the period* 13 *May–*4 *July* (*figures in brackets show percentage of total*)

| Trap type | *A. sputator* | *A. obscurus* | *A. lineatus* | Total |
|---|---|---|---|---|
| 1 | 67·2 (37) | 102·5 (57) | 10·5 (6) | 180·2 |
| 2 | 41·2 (36) | 57·8 (51) | 14·2 (13) | 113·2 |
| 3 | 41·2 (36) | 65·0 (57) | 8·5 (7) | 114·7 |
| 4 | 41·9 (34) | 70·4 (58) | 9·7 (8) | 122·0 |
| 5 | 55·0 (44) | 58·3 (47) | 10·7 (9) | 124·0 |
| 6 | 26·3 (31) | 52·3 (62) | 6·0 (7) | 84·6 |
| 7 | 18·0 (32) | 33·5 (59) | 5·0 (9) | 56·5 |
| 8 | 15·5 (26) | 36·5 (61) | 7·5 (13) | 59·5 |
| 9 | 32·0 (37) | 47·5 (56) | 6·0 (7) | 85·5 |
| 10 | 14·5 (35) | 21·5 (51) | 6·0 (14) | 42·0 |
| | 352·8 (35·9) | 545·3 (55·5) | 84·1 (8·6) | 982·2 |

ploughed up. Salt & Hollick (1944) showed that the hand-sorting method was likely to indicate a population based on the last three instars only, and if it is assumed that the above reduction is in the main due to the emergence of adults it should be possible to estimate the wireworm population on this basis by multiplying the number of adults per acre by three. Thus for the 1944 field the figure would be 1,437,480, which is very near the figure 1,577,000 obtained by counting the wireworms. In 1946 the peak figure was eight (Table 1), i.e. 348,480 per acre. Multiplied by three this gives 1,145,440, which is near the figure 1,100,000 given by a wireworm count.

The question arose as to whether the traps attracted the beetles and thus gradually exhausted the population over a limited area, or whether they really registered a local movement. In an attempt to answer this, traps were arranged in pairs 1 yd. apart; the beetles were removed from one trap of each pair (*a*) and replaced in the other (*b*). In 1944 ten pairs of traps were operated from 15 to 20 June, the yields for fifteen examinations being (*a*) 114 and (*b*) 179. In 1946 the beetles in certain traps were counted and replaced. The figures for six consecutive days, 6–11 June, were 48, 47, 42, 47, 45 and 47. It would seem, therefore, that there is no accumulation of beetles over a period and that traps of type 1 do not attract, but merely shelter, beetles which enter in the course of their wanderings. That there might be a tendency to shelter at certain times of the day is shown later when discussing periods of activity.

*Molasses.* In 1946 four traps were placed on the ground, each consisting of a biscuit-tin lid coated with molasses and a sprinkling of grass clippings. They did not attract any beetles until 31 May, after which date the numbers caught gradually rose. By 4 July twenty-one *A. lineatus*, 197 *A. obscurus* and eighty-seven *A. sputator* had been caught. These figures show that the three species were caught in proportions similar to those counted in traps 1–10 of the hay and grass types.

Most of the beetles caught on the molasses traps were lying on their elytra at the edge of the molasses but not in contact with it. There was no evidence of their having been entangled, and it would seem that they were merely intoxicated by feeding. The average total yield per trap for the period 13 May–4 July was 76·3. It varied in efficiency according to weather conditions, chiefly rain and sunshine.

## Periods of activity

*Annual activity above ground.* The preliminary work in 1944 was carried out between 6 June and 7 July and showed that *Agriotes* spp. were active over the whole period. Large numbers were found on the bottom of a hay cart in this field on 10 July. Egg-laying by beetles in captivity commenced about 24 June in all three *Agriotes* species, and mortality amongst these captive beetles had reached high proportions by the end of June. Many beetles were found dead and some partially eaten during the last week in June in both years.

In 1946 a negligible number of beetles was caught before 20 May (Table 1). After that date numbers rose rapidly as shown in Fig. 2.

From 24 to 27 May 1946, a number of beetles, light coloured as if freshly emerged, were collected from the traps. These included all three species under consideration, but after the latter date, the beetles found in this condition were a few *A. sputator* daily until 12 June and an occasional single *A. obscurus* on 30 May, 4, 6 and 17 June.

Figs. 1 and 2 show the average daily catch in trap type 1 in 1944 and 1946. In both years it would appear that peak activity was soon after emergence towards the end of May; this was followed by a gradual decline in activity until about 20 June, after

which date a second, though small, peak of activity occurred. This second peak coincided with egg-laying by beetles in captivity and it occurred in all three species.

Fig. 3 shows the number of adults (expressed as a percentage of the number of larvae) found in soil samples from fields sampled in the Midland Province by the wireworm survey teams from 1942 to 1945. It will be seen that the number of beetles found in the samples during June is very low; this coincides with the time of maximum activity above ground.

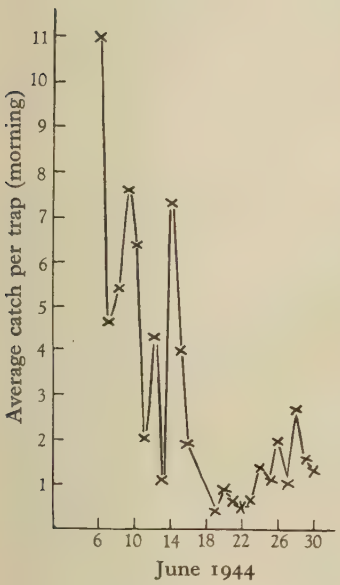


Fig. 1. Daily average catch of *Agriotes* spp. in hay traps (type 1) during June 1944.

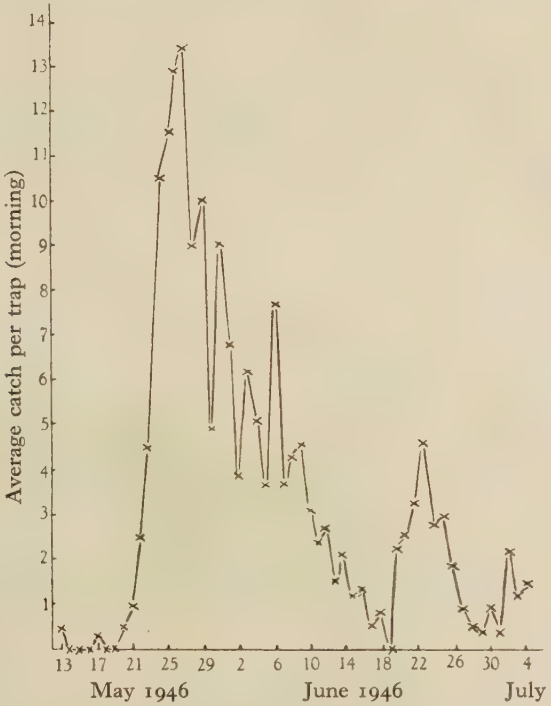


Fig. 2. Daily average catch of *Agriotes* spp. in hay traps (type 1) during summer 1946.

In 1946 traps were placed in the field again from 25 August to 2 September but no beetles were caught.

*Daily activity*. In 1944 the traps were frequently examined at different times of the day, although the morning counts have been used as the standard in this paper. Usually a maximum number of beetles was found in the traps in the early morning, i.e. between midnight and 6 a.m. G.M.T. A minimum number was found in the afternoon and evening. Fig. 4 shows in graphical form the records per trap of type 1 over a period of 4 days. The fluctuation in numbers trapped probably reflects

a temperature effect, the beetles being most active during the afternoon and evening when the grass temperature is at a maximum, and seeking the shelter of the traps during the early morning when the grass temperature is at a minimum. On the night of 16–17 June the maximum number of beetles was found somewhat earlier than usual, and records taken at a nearby meteorological station showed that there had been only 1 hr. of sunshine during the previous day; this and the fact that there was a cool north-west wind blowing during most of the day may have induced the beetles to seek shelter earlier than usual. In 1946, if the traps were examined in the

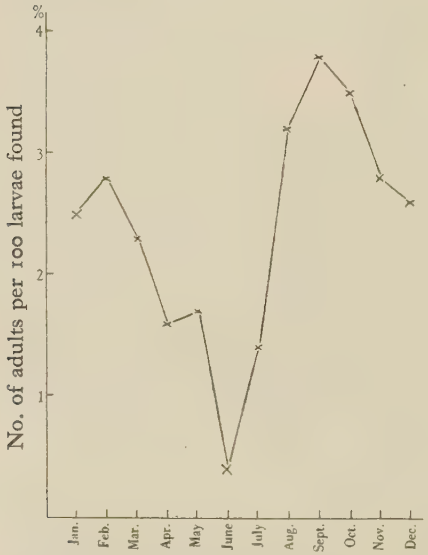


Fig. 3. Number of adults per 100 larvae found in soil samples from fields sampled by the wireworm survey teams of the Midland Province. Average figures for the years 1942–5.

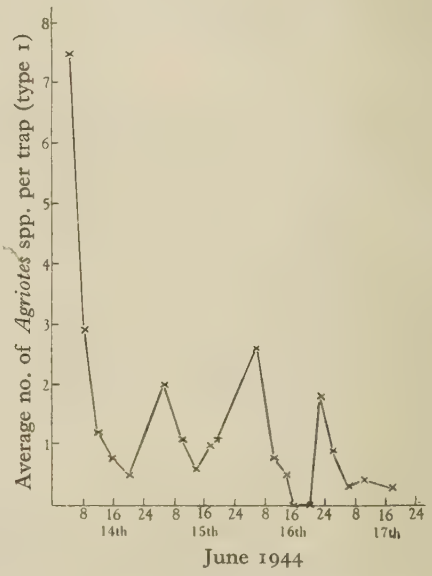


Fig. 4. Average number of *Agriotes* spp. per trap (type 1) at different times of day.

afternoon, after being cleared in the morning, there was approximately one-third of the morning population present. Cohen (1942) found that movements took place chiefly during the night.

Attempts to correlate variations in daily activity with meteorological conditions were not successful. In 1944 numbers fell rapidly from 15 to 22 June. This coincided with a hot, dry period which lasted until 25 June. However, a similar fall in numbers occurred in 1946 when hot, dry conditions were not experienced. On 22 June 1944, when the ground had become very dry, thirty traps of type 1 were laid, ten with the normal 3 in. thickness of hay, ten with a 6 in., and ten with a 3 in. depth but with

the hay and ground beneath liberally watered. The total numbers of *Agriotes* spp. taken in two examinations during the day were:

| | |
|---|---|
| Normal | 7 |
| Double thickness of hay | 9 |
| Normal, watered | 15 |

It would seem, therefore, that the beetles definitely preferred the more humid conditions.

### Cover preferences

*Herbage height.* The height of the herbage in the poor meadow used in 1944 varied greatly. Traps were situated as follows: seven amongst short herbage about 3 in. high, eight amongst medium herbage about 7 in. high and five amongst tall herbage about 1 ft. high Catches from these traps, expressed as percentages of the total catch, over two periods were:

| Herbage | Short | Medium | Tall |
|---|---|---|---|
| 15–21 June | 20 | 17 | 63 |
| 22 June–1 July | 38 | 31 | 31 |

A marked preference for traps amongst tall herbage was shown during the earlier period, coinciding with a week of high winds and no rain, and it is possible that the greater humidity at the base of tall herbage under these conditions encouraged greater movement. The later period was calm and wet, and under these conditions no preference for tall herbage was shown.

A similar effect was noted when ten traps of type 1 were placed in an adjoining pasture. The catches over 2 days (15–16 June) averaged 0·6 *Agriotes* spp. per trap in the pasture and 4·3 in the meadow.

*Site in field; effect of hedges.* In 1944 five traps of type 1 were placed near the hedge, two under the hedge, one at 1 yd. and two at 4 yd. from the hedge. These yielded in the period 20–30 June:

| | Average no. beetles per trap |
|---|---|
| Under hedge | 3 |
| 1 yd. distant | 7 |
| 4 yd. distant | 18 |

In 1946 numerous traps were placed under the hedges, at 2–8 yd. from the hedge (referred to as 'near hedge') and in mid-field. The total average yield of trap type 1 in these situations over the period 13 May–4 July was:

| | |
|---|---|
| Under hedge | 2·5 |
| Near hedge | 148·9 |
| Mid-field | 193·1 |

These figures show that the shelter afforded by hedges had no attraction for the three common species of *Agriotes*. Evidence from the average daily yield per trap near the hedges and in mid-field suggested that emergence of the beetles might have

been slightly earlier near the hedges. Table 3 gives the daily total of *Agriotes* spp. caught in all traps during the latter half of May 1946.

TABLE 3. *Total* Agriotes *spp. caught in all traps* 21–31 *May* 1946

| | Near hedge | | Mid-field | |
|---|---|---|---|---|
| May | Total beetles | No. of traps | Total beetles | No. of traps |
| 21 | 6 | 13 | 8 | 16 |
| 22 | 25 | 13 | 19 | 16 |
| 23 | 23 | 13 | 13 | 16 |
| 24 | 163 | 21 | 20 | 16 |
| 25 | 236 | 21 | 29 | 16 |
| 26 | 242 | 21 | 60 | 16 |
| 27 | 269 | 21 | 106 | 16 |
| 28 | 158 | 21 | 101 | 20 |
| 29 | 97 | 21 | 138 | 20 |
| 30 | 91 | 21 | 82 | 20 |
| 31 | 144 | 21 | 204 | 20 |

TABLE 4. *Captures of marked* Agriotes obscurus *released at the centre of a series of concentric circles on* 13 *June* 1946

| Distance from centre in ft. (figures in brackets show no. of traps) ... | 3 (4) | 6 (2) | 9 (2) | 12 (2) | 15 (2) | 18 (2) | 24 (2) | 27 (2) | 36 (2) |
|---|---|---|---|---|---|---|---|---|---|
| 14 June | 2 | . | . | . | . | . | . | . | . |
| 15 | 10 | . | . | . | . | . | . | . | . |
| 16 | 18 | 1 | . | . | . | . | . | . | . |
| 17 | 6 | . | . | . | . | . | . | . | . |
| 18 | 15 | 2 | 2 | 2 | . | . | . | . | . |
| 19 | 9 | 1 | 2 | 1 | . | . | . | . | . |
| 20 | 8 | 4 | . | . | . | . | . | 1 | . |
| 21 | 6 | . | . | . | . | . | . | . | . |
| 22 | . | 1 | 1 | . | . | . | . | . | . |
| 23 | . | . | . | . | . | . | . | . | . |
| 24 | 1 | . | . | . | . | 1 | . | . | . |
| 25 | . | . | 1 | . | . | . | 1 | . | . |
| 26 | . | . | . | . | . | . | . | . | . |
| 27 | 1 | . | . | . | . | . | . | . | . |
| 28 | . | . | . | 1 | . | . | . | . | . |
| 29 | . | . | . | . | . | . | . | . | . |
| 30 | . | . | . | . | . | . | . | . | . |
| 1 July | . | . | . | . | . | . | . | . | . |
| 2 | . | . | . | . | . | . | . | . | . |
| 3 | 2 | . | . | . | . | . | . | . | . |
| 4 | . | . | . | . | . | . | . | . | . |

## MOVEMENT OF MARKED BEETLES

Beetles were marked by small spots of cellulose paint on their elytra.

In 1944 twenty *A. lineatus*, twenty-five *A. obscurus* and fifty *A. sputator* were marked and released on 20 June in three central traps, the markings being different

for each trap. Twelve other similar traps of type 1 were scattered around the central ones at distances varying between 15 and 30 yd. Two days later eighteen marked beetles were removed from the traps in which they were released, and records were taken until 30 June of marked and unmarked beetles in the traps. Eight *A. lineatus*, three *A. obscurus* and six *A. sputator* were recovered from the same traps in which they had been released. One *A. sputator* was recovered in a trap 26 yd. away 11 days after release; 130 more marked *A. sputator* were released in two other traps on 25 June, and during the following 5 days forty-seven of the beetles re-entered the traps in which they had been released.

In 1946, 448 marked *A. obscurus* were released on 13 June in one place in a meadow and traps placed on concentric circles at intervals of 3 ft. around the point of release at distances from 3 to 36 ft. The traps were examined once daily until 4 July and 100 marked beetles were recovered. Details of catches are shown in Table 4.

A total of 100 beetles was recovered from 448 released. There was no evidence that the beetles showed a preference for any particular direction of movement.

## Conclusions

The following observations are based on the trapping of 7737 adults in the two seasons.

The period of activity above ground of the three chief pest species of *Agriotes*, namely *A. obscurus*, *A. lineatus* and *A. sputator* extends from the end of May to early in July.

Movement is almost entirely along the ground or on low plants, although flighting has been recorded on rare occasions by other investigators.

While some of the beetles may travel many yards in a few days, the majority make either very short journeys or move in a very restricted area. Probably their movements are so haphazard that whatever distances they travel their position alters little from day to day.

Traps merely hold the beetles and do not attract them; hence as a control measure their efficiency depends on the area covered.

It would seem that each field is a self-contained habitat. Hence it is probable that an arable field put down to grass will be dependent on its own individuals for gradually building up a high population.

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(*Received* 30 *October* 1946)

# OBSERVATIONS ON THE LIFE CYCLE OF *XYLEBORUS FORNICATUS* EICHOFF IN ARTIFICIAL CULTURE

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(With 1 Text-figure)

The scolytid beetle, *Xyleborus fornicatus* Eichoff, which is an important pest of the tea bush in Ceylon, has been reared from egg to adult for the first time in artificial culture with its ambrosial fungus *Monacrosporium ambrosium*. Deaths in culture were numerous, yet sufficient data on the periods occupied in the different stages were collected to afford an opportunity for testing conclusions derived by the use of less direct methods. Results indicate that temperature materially affects the length of the life cycle and mainly controls the altitudinal distribution of the pest in Ceylon.

## Introduction

The duration of the various stages in the life cyle of individual scolytid beetles has rarely been determined precisely, because of the difficulty of breeding them under conditions allowing of direct observation. *Xyleborus fornicatus* Eichoff has been known as a pest of tea in Ceylon since 1893, and the first account of its life history and habits was published by Green in 1903. Since then much has been written about the insect as a pest, but little has been added to Green's account of its life history and habits.

Table 1. *Previously published records of duration (in days) of stages in the life history of* Xyleborus fornicatus

| | Speyer (1917*a*) | King (1937) | Gadd (1941) |
|---|---|---|---|
| Entrance of female to laying of first eggs | 25 | 10 | 8·4 |
| Egg stage | 4 | 4 | 6·9 |
| Larval stages | 25 | 13 | 15·2 |
| Pupal stage to emergence of adult | 6 | — | — |
| Pupal stage | — | 7 | 7 |
| Adult before emergence | — | — | 2–3 |
| Total | 60 | 34 | 40·0 |

Speyer (1917*a*) published data, given in Table 1, concerning its life history at an altitude of 4000 ft. above sea-level, but without stating how the information had been obtained. In the same table are given data published by King (1937) after opening and noting the contents of galleries of known ages, and those of the writer (1941), probably derived from the same data as used by King, and collected at an altitude of 3500 ft., using graphical methods which took into account all the original observations.

A glance at the table will indicate that there is little agreement regarding the duration of any stage. Although none of the different stages is likely to be constant under different conditions, it is likely that divergences shown are due mainly to the methods of estimation. That is almost certainly true as regards the differences between King's and Gadd's results. For this reason and because accurate data are required for the interpretation of population censuses, an attempt was made to culture the insects through all stages under conditions allowing of direct observation.

## EXPERIMENTAL

*Pre-oviposition.* The female beetle has to bore a new gallery before starting to lay eggs. This takes several days, but the cessation of boring is not indicative of the beginning of oviposition. King found no eggs in galleries less than 10 days old. Galleries of much greater age also may not contain eggs or young, e.g. the writer found 10% of inhabited galleries 4 and 5 weeks old to contain no eggs or young, and it is possible, even probable, that some beetles may bore galleries yet never lay eggs. Naturally, egg-laying starts during the second week. Of fifty-two galleries opened, 8–14 days old counting from the commencement of the gallery, twenty-five contained eggs, totalling in all seventy-five. These figures suggest that Speyer's estimate of 25 days is excessive for the normal preoviposition period, and that King's estimate of 10 days is tolerably correct.

*Incubation period.* Oviposition has not been observed as it has not been possible to induce female *X. fornicatus* to lay eggs outside the galleries. Eggs for experimental purposes have therefore to be obtained by opening galleries. There they occur in varying number, often in small clumps of five or six, which may give rise to the view that eggs are laid in batches. The eggs will hatch normally in moist chambers, and the order in which the eggs hatch is indicative of the order in which they were laid.

In Table 2 are given data of the hatching of eggs, collected from galleries in tea and castor-oil stems, when placed under different temperature conditions. The eggs collected were, of course, of unknown ages, but those which took longest to hatch were undoubtedly the youngest. At room temperature (mean 72·6° F.) at Passara (altitude 3500 ft.) only one egg from tea galleries took 9 days to hatch, and four required 8 days. Very similar results were obtained with castor eggs in the same laboratory with a slightly higher mean temperature. Three hatched in 8 days and thirteen in 7 days. At St Coombs (altitude 4550 ft.) eggs from a castor stump were placed in an incubator at 28° C. (82° F.). The incubator was electrically controlled, but as power was shut off for some hours at night the temperature gradually fell until power was switched on again a few hours later. Under these warmer conditions the incubation period was shortened to 5 or 6 days.

It is evident, therefore, that both Speyer's and King's estimates for the egg stage (4 days) are much too short for the altitude their data refer to. At 3500 ft. the incubation period would rarely be less than 7 or more than 8 days.

The data relating to individual galleries in Table 2 are given as typical of the many recorded. They show that rarely do as many as three eggs of any one clump hatch on any one day; one is the more usual number. This indicates that although the eggs are grouped together they are not laid as a batch at one time. The females normally lay one egg a day, occasionally two and rarely three. On some days no egg is laid.

During the last few years a very large number of galleries in tea branches have been opened and their contents counted. Galleries such as A and B in Table 2 contain exceptionally large numbers of unhatched eggs, ten being the maximum

TABLE 2. *Time of hatching of* Xyleborus fornicatus *eggs after removal from galleries to damp chambers*

| Gallery | No. of eggs | Day of hatching 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Ex* tea. Passara Laboratory: room temperature 67–78° F.; mean 72·6° F. | | | | | | | | | | |
| A | 10 | 1 | 1 | 1 | 2 | 1 | 1 | 3 | — | — |
| B | 9 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | — | — |
| C | 5 | 1 | 1 | 1 | 2 | — | — | — | — | — |
| D | 5 | 1 | 1 | 1 | — | — | 1 | — | 1 | — |
| Many | 92 | 15 | 16 | 8 | 11 | 14 | 13 | 11 | 3 | 1 |
| Total | 121 | 19 | 21 | 13 | 16 | 16 | 16 | 15 | 4 | 1 |
| *Ex* castor. Passara Laboratory: room temperature 70–82° F.; mean 75·6 F. | | | | | | | | | | |
| E | 6 | — | 1 | 2 | 1 | 1 | — | — | 1 | — |
| F | 6 | 1 | 3 | 1 | 1 | — | — | — | — | — |
| G | 4 | — | — | — | 1 | 2 | 1 | — | — | — |
| Many | 185 | 19 | 26 | 38 | 37 | 22 | 28 | 13 | 2 | — |
| Total | 201 | 20 | 30 | 41 | 40 | 25 | 29 | 13 | 3 | 0 |
| *Ex* castor. St Coombs Laboratory: incubator 28° C. (82° F.) | | | | | | | | | | |
| H | 7 | 1 | 3 | 1 | 1 | 1 | — | — | — | — |
| J | 7 | 2 | 1 | 2 | 1 | 1 | — | — | — | — |
| K | 6 | 1 | — | 1 | 2 | 1 | 1 | — | — | — |
| Many | 45 | 13 | 10 | 8 | 7 | 5 | 2 | — | — | — |
| Total | 65 | 17 | 14 | 12 | 11 | 8 | 3 | — | — | — |

found. Usually the number is less than seven. The reason for this becomes apparent when the rate of egg-laying and the incubation period are taken into account.

*Larval period.* Determination of the exact duration of the larval stages of scolytid beetles by direct observation is dependent on an ability to maintain the insects alive outside their normal galleries under conditions allowing them to complete development. The main difficulty in the past appears to have been the provision of proper food. *X. fornicatus* is an ambrosial beetle whose larvae feed upon a fungus, *Monacrosporium ambrosium* (Gadd & Loos, 1947), which grows on the gallery wall. It also grows freely on nutrient agar. When larvae are transferred from freshly opened galleries to such cultures in tubes, they begin to feed immediately, and many, in time, pupate.

Pure cultures of the ambrosia fungus were obtained from galleries in tea and

castor stems. These differed in certain minor characters, but the larvae, whether from tea or castor galleries, fed as readily on one strain as on the other, and no observable differences in growth resulted. No further mention need therefore be made of the particular strain of fungus used in the different experiments.

In order to determine the full larval period, eggs from tea and castor galleries were hatched on damp blotting paper at St Coombs. The tea eggs were sent from Passara by post, but the castor eggs were obtained from sections of cut stems maintained in the St Coombs laboratory. Newly hatched larvae were placed in tube cultures of the ambrosia fungus on nutrient agar slants. The number placed in the tubes varied, depending on the number available that day, but never more than twenty were put in a $6 \times \frac{3}{4}$ in. culture tube. The larvae began to feed immediately they were placed on the fungus, and continued, apparently unceasingly, for the first 2 or 3 days, when many became quiescent preparatory to moulting. At this stage and during the following few days deaths were very numerous, but after the first moult the larvae normally thrived until due for pupation.

The full larval period varied considerably with temperature. With cultures kept in the incubator at 28° C., the shortest period observed was 9 days for both tea and castor larvae, and the longest period was 16 days for tea and 19 days for castor larvae. The variation in the larval periods observed, however, cannot be attributed to the fall in temperature at night as the larvae in any one tube rarely pupated at the same time. Many larvae, although full grown, did not pupate at all but continued to feed, often till the 30th day before dying.

The following is typical of what occurred in numerous experiments. Sixteen larvae newly hatched from eggs collected from castor galleries were placed in a tube in which the tea ambrosia was growing. Three days later five had moulted, five were quiescent, five were still feeding but still in the first stage, and one was dead. On the sixth day, five had just entered the third and final larval stage, three were in the second stage, three still in the first stage, quiescent, but four more in the first stage were dead. The first pupa was seen on the 10th day. One larva pupated on each of the following 4 days, leaving on the 14th day five well-grown larvae in the tube. None of these pupated and the last three deaths occurred on the 27th day. Of the sixteen larvae originally placed in the tube only five pupated. Other tubes gave an even smaller number of beetles. Failure was due largely to the heavy mortality at the first moult and to many larvae failing to pupate at the due time. Obviously culture conditions were not ideal, but attempts to improve them, as by frequent transfer to fresh cultures, were not successful.

No notable difference was observed between the larval periods of the tea and castor insects, nor did the source of the ambrosia fungus appear to affect the period. The mean larval period of thirty-one insects at 28° C. was 12·4 days. Very similar results were obtained when the larvae were incubated at 25° C., with a fall in temperature at night for a few hours. The optimum temperature appears to lie between 25 and 28° C.

At room temperature at the St Coombs Laboratory (altitude *c.* 4500 ft.) deaths were still more frequent, and the larval period longer. The temperature fluctuated between 16 and 25° C. with a mean daily temperature of about 20° C. The shortest larval period observed was 18 days and the longest 33 days. The mean of eight larvae was 23·6 days. Some larvae lived as long as 40 days without pupating.

For one or two days before pupation the larva lies on its back and becomes quiescent. In the foregoing observations this prepupal period has been counted as part of the larval period. At room temperature full-grown larvae were sometimes found on their backs preparatory to pupation and they died later in that position without pupating. Similar occurrences were not observed in the incubator cultures.

As the length of the larval period is affected very materially by temperature it may be inadvisable to offer comment on the values shown in Table 1. It would appear, however, that Speyer's estimate of 25 days is perhaps excessive although made at 4000 ft., as it exceeds the average determined at room temperature at St Coombs situated at an altitude at which the beetle cannot maintain itself in the adjoining tea

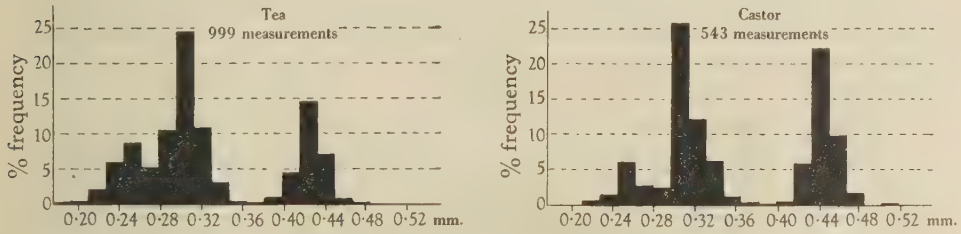


Fig. 1. Measurements of widths of head capsules of *Xyleborus fornicatus* larvae from tea and castor galleries plotted in frequencies of successive multiples of 0·017 mm.

fields. King's estimate of 13 days approximates to the average (12·4 days) obtained from cultures grown at 28° C. and is possibly too low a value for beetles living at an altitude of 3500 ft. at which his data were collected. The writer's own estimate of 15·2 days is probably nearer the truth.

*Instars.* There are three stages in the development of the larva. This fact can of course be determined by measuring the head capsule widths of larvae of unknown ages taken directly from galleries. In Fig. 1 are histograms representing the measurements of two such collections, one of 999 larvae from tea galleries and the other of 543 from castor galleries. The frequencies are shown as percentages of each collection. The measurements were recorded in categories differing by 0·017 mm., the length corresponding to one unit of the eyepiece micrometer used.

Both histograms show three peaks, the first at 0·255 mm., the second at 0·306 mm. and the third at 0·425 mm. for tea and at 0·442 mm. for castor. That the apparent peak at 0·255 mm. for tea larvae is in fact a true peak can be demonstrated by measurements made of newly hatched larvae. Of forty-six such larvae, twenty had head-capsule widths of 0·255 mm., eighteen of 0·238 mm. and six of 0·221 mm.

None measured more than 0·255 mm. It will be evident therefore that *Xyleborus* larvae from tea galleries have head capsules measuring 0·180–0·272 mm. while in the first stage, 0·272–0·374 mm. in the second stage, and over 0·374 mm. in the third stage.

There is less doubt about the peak at 0·255 mm. in the histogram of castor beetles, and measurements of newly hatched larvae confirm it. Of eighty-three such larvae measured, only three had head capsules exceeding 0·255 mm. in width. The head capsules of first, second and third stage larvae from castor measure 0·214–0·272 mm., 0·272–0·382 mm. and over 0·382 mm. respectively. These measurements and the histograms indicate that castor-beetle larvae tend to be slightly larger than tea-beetle larvae, particularly in the third stage. This perhaps is not entirely surprising, as typically adult castor beetles are slightly larger, on the average, than beetles from tea.

If the larvae are classified according to the stage of development reached, it can be ascertained that of the tea population approximately 200, 520 and 279 larvae were in the first, second and third stages respectively. The corresponding figures for the castor population are 52, 271 and 220. In both instances the smallest number was found in the first stage, owing to the fact that the first stage occupies the shortest period.

A question which may here be considered is whether the period of time occupied in any one stage of development is proportional to the number found in that stage. Such an hypothesis can be valid only if the insect is not seasonal and there is an inextricable overlapping of generations. The climatic conditions for the period preceding the sampling should be fairly uniform, and only few deaths should occur during development and these distributed uniformly over the instars. Also the sample should be representative of the whole population.

Expressing the numbers found in the different stages of development as ratios the following are obtained: 1:2·6:1·4 for the first, second and third stage larvae from tea and 1:5·2:4·2 from castor. These figures suggest that castor beetles spend relatively a longer time in the second larval stage than do tea beetles. This conclusion can, however, be tested.

Mention has already been made of the great variation in the length of the full larval period observed in artificial cultures under controlled conditions. No attempt was made to determine the mean instar periods accurately as that would entail culturing the insects separately. What was noted was the first appearance of the different stages in the tubes. For instance, in one tube containing tea larvae on tea ambrosia growing at 28° C. the first larva to reach the second instar was observed at the end of the 2nd day, and the first to start the third stage was noted at the end of the 6th day; the first pupa in the same tube was seen on the 10th day. It is probable, though not certain, that these observations refer to the same insect, in which case the ratio of the instar periods can be written 1:2:2. Exactly similar results were obtained from tubes containing castor larvae feeding on tea ambrosia at the same temperature.

These observations, supported by others of a similar nature, indicate that there is little or no difference in the relative lengths of the different instar periods between larvae from tea and castor when grown under artificial conditions. It is probable therefore that no great reliance can be placed on the differences between the two life cycles noted when the instar period ratios were calculated from the numbers counted in each instar.

The relatively short period determined for third stage larvae by that method suggests that in nature larvae do not continue feeding for long periods after the normal period for pupation, as was observed in the artificial cultures.

*Pupal period.* The pupal period can be determined in the same way as the incubation period by the use of moist chambers. Beetles emerged from pupae of unknown ages, collected from freshly opened tea galleries and incubated at room temperature at the Passara laboratories, on the 8th day or earlier. The data as a whole indicated clearly that under those conditions 8 days is the normal pupation period. Similar results were obtained with castor pupae.

At St Coombs, pupae were obtained from cultures on the day of pupation and so could be observed during the whole pupation period. At 28° C. the pupal period of tea beetles varied from 4 to 6 days, with a mean of 5·2 days. Pupae derived from castor eggs exhibited a similar variation giving a mean of 5·3 days. Similar results were obtained when the pupae were incubated at 25° C., but at room temperature the period extended from 9 to 11 days giving a mean of 9·9 days.

Speyer's estimate of 6 days is undoubtedly too short for the pupal period, especially as he included with it a period elapsing before the adult emerges from the gallery. King's estimate of 7 days, for the pupal stage alone, is approximately correct and in fair agreement with the result obtained at room temperature at Passara (8 days).

*Adult colour.* The adults at first are pale yellow with black mouthparts and eyes. Females have their wings also, but not the elytra, dark coloured. Male bodies having no true wings appear entirely yellow except for the eyes and mouthparts. These coloured parts become apparent in the pupa 2 or 3 days before the adult emerges.

Adults leaving galleries in the field are entirely black, but little is known about the time taken for full colour to develop. Green was of the opinion that 'after some hours the full colour is assumed', and Speyer (1917*b*) held a similar opinion, putting the period as 'a few hours'. In cultures, colour development is rather slow, and at least 5 or 6 days will elapse before the beetles are fully black. A delay in colour development may result from cultural conditions though a reason is not obvious. It is certain, however, that the colour is not a matter merely of a few hours, but that some days elapse before the newly emerged adult leaves the gallery. That fact accounts for the accumulation of young beetles found in galleries.

*Mating.* The male to female ratio of beetles in tea stems is 1 : 4 or 5, and in castor stems it is probably much wider. Green and Speyer agree that mating occurs within the gallery, and such evidence as there is points to that conclusion. The main difficulty in accepting it is an inability to understand how fertilization can occur

within the gallery, because the tunnel is of such diameter that although a female may move freely along it, it is insufficient to allow one female to pass another. It is doubtful, too, whether the smaller male could pass a female within the gallery. Also there are no special places of greater diameter or height which might serve as nuptial chambers.

The problem is not simplified by a statement by Speyer that 'as these (males) are ejected by the female they are not common in normal galleries'. In laboratory cages males are frequently seen walking on the stems, and they have been observed to follow young females into galleries recently excavated. It cannot be suggested, however, that copulation takes place in the new galleries, as the same objections must be raised to that locality as to the old gallery.

The males from castor stems have been observed in the laboratory to excavate chambers adjoining gallery entrances and to lie in them as though 'in wait'. Not all males do so, nor does every gallery entrance have a 'male cavity' adjoining it. In one instance as many as four cavities surrounded a gallery entrance. These observations suggest that copulation may occur at gallery entrances, but such has not been observed. Similar cavities have not been found at gallery entrances in tea stems.

Mating has not been observed in cultures, and there is doubt that females have ever been fertilized under those conditions.

*Humidity requirements.* When adults are removed from galleries and placed in tubes with dry blotting paper they continue to live for 2 or 3 days only. If the blotting paper is kept moist some of the beetles live as long as 15 days. Similar results are obtained when sawdust is used instead of blotting paper.

Evidently adult beetles require humid conditions for survival, and the time they can survive outside a gallery is very limited during normal weather conditions. For the same reason it is unlikely that females will undertake long flights. In the laboratory the beetles from tea show no inclination to fly at all, although castor beetles are more active at times. A young female will leave the parent gallery, walk along the stem and begin her own gallery a short distance away, or she may move to another stem in close proximity. Others will wander about the stems without boring until they die. Why some will bore and others will not, under identical conditions, is not apparent. A female will bore a tunnel in a tea stem equal to her own length in about 1 hr.

*Culture adults.* Although beetles raised entirely in artificial culture have been kept alive for as long as 30 days, few have been induced to bore into cut stems, and then the galleries made have been short and incomplete. No eggs have been laid by any female so raised, either inside or outside a gallery, even when a male has been maintained with her.

## Discussion

Many insects, particularly scolytid beetles, and other organisms, e.g. parasitic nematodes, spend the greater part of their lives in obscurity, hidden within the tissues of their host, which renders study of their life histories most difficult. A method first used by the writer (Gadd & Loos, 1941) for determining the life history of a parasitic nematode (*Pratylenchus* (*Anguillulina*) *pratensis*) was later adopted for use with the beetle *Xyleborus fornicatus* with the results given in Table 1. The fact that the beetle can be grown and kept under direct observation in artificial culture with the appropriate ambrosia fungus has afforded a means of verifying previous conclusions and so testing the accuracy of the indirect method of study. The present investigations show that the calculated periods of the various stages in the life cycle of *X. fornicatus* are substantially correct, and that although the previous estimate for the full cycle (40 days) is an underestimate, the error is at most a few days.

Variation of temperature has a marked effect on the duration of various stages, particularly the larval stage, and consequently on the full life cycle of *X. fornicatus*. At room temperature at St Coombs Laboratory deaths were extremely numerous and the life cycle so prolonged that the suggestion of such temperature being close to the minimum for the insect appears justifiable.

The distribution of the insect in Ceylon is limited by altitude. Speyer, writing in 1918, observed that the borer becomes less common when 4000 ft. is reached, and above 4500 ft. it is no longer a serious pest. That account of its distribution is equally true to-day. St Coombs Laboratory is situated at an elevation of about 4500 ft. above sea-level, and the surrounding tea is practically free of the pest. At times a few galleries are found when the bushes are pruned but they are usually unoccupied by beetles. These observations indicate that although beetles reach that elevation, make their galleries and probably start breeding, they are unable to establish themselves permanently there. As the indoor temperature at night is somewhat higher than the outdoor, which at times falls almost to freezing-point, it seems justifiable to conclude that the altitudinal distribution of the beetle is controlled mainly by temperature.

*Maternal care.* Mention has been made of the abnormally large number of deaths in artificial cultures and of the failure of many full-grown larvae to pupate. In natural galleries the young are always tended by the mother, but what she does apart from protecting the gallery against invaders, and possibly keeping the gallery clean, is not known. In cultures the young were not so tended, although larvae have been observed to clean others by removing fungus adhering to their heads and bodies. Deaths occur in natural galleries and, as estimated elsewhere, may amount to as much as 50% of the eggs laid. At what stage such deaths occur is unknown, but from the culture experiments it would appear most probably to be at the first moult.

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(*Received* 22 *November* 1946)

# THE PROTECTIVE EFFECT OF ARSENIC AND DERRIS DIPS AGAINST THE SHEEP TICK, *IXODES RICINUS* L.

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(With 7 Text-figures)

Sodium arsenite used at a bath strength of 0·2% $As_2O_3$ is shown to protect sheep to a large extent from infestation for periods of from 1 to 2 weeks. Coal-tar creosote dips, though usually effective in killing attached ticks, have no protective effect against reinfestation. Derris, either as ground root or as extract, kills all attached ticks at dilutions down to the order of 1 part extract in 15,000 of water. At a concentration of 1 part in 5000 it has an effective duration comparable to that of arsenic at 0·2% $As_2O_3$. Its maximum effective duration, when applied as an emulsion wash, appears to be 14–17 days.

The addition of wool grease enhances the effective duration of arsenic, but not of derris.

The effect of immersion time is important for both arsenical and derris dips, but is especially important for the latter. For full effect with derris washes sheep must be immersed for at least half a minute.

A protective dip is described which markedly lowers the infestation of dipped sheep for about 3 weeks.

## Introduction

Dipping of cattle against ticks, which was popularized by pioneer work in the United States and South Africa at the close of last century and the early years of this, is to-day the common practice in every cattle-raising country where ticks exist. The method has received the attention of scientists throughout the world; the classic work of Watkins-Pitchford in South Africa and of Ransom and Graybill in North America, and, more recently, the work of the Queensland Cattle Tick Committee in Australia, are but the more outstanding of many notable contributions. Almost all of the recorded work refers to the control of ticks on cattle, and among all workers there is complete agreement that arsenic forms the most satisfactory tick-killing substance. It has been customary to consider the action of arsenic purely from the point of view of efficacy in killing those ticks attached at the time of dipping; the protective effect of dippings against later infestation was regarded, and with justice, as unreliable and of very short duration.

It is not surprising, therefore, that when, in recent years, the question arose of controlling tick infestation of sheep, the investigators should start with a natural bias towards the use of arsenic in dip form and should also follow the custom of considering only the immediate killing effects of the dip, disregarding the possibility of protection. The first attempts to control the sheep tick, *Ixodes ricinus* L., appear to have been made by this Bureau at Kielder, Northumberland (unpublished work),

and in north Wales (Walton, 1927). In both instances a short-interval dip was used, and the reduction in numbers of attached ticks on the dipped sheep was attributed to a reduction of the available ticks as a result of the dippings.

Short-interval dipping of sheep as a measure of general application in this country is handicapped by the fact that hill stocks are almost invariably breeding stocks, and the tick season corresponds with their lambing season. Further, the stocking of hill ground is so light, sometimes no more than one sheep to 5 acres, that frequent gathering, over the many miles of rough mountain and moor, is impracticable.

In 1933, certain observations made in the field led me to suspect that the effect of arsenical dips against ticks on sheep might be considerably more lasting than was generally accepted, and that sheep might be protected from ticks by dipping. During the years 1933–8 I carried out much laboratory and field work on the question, some of the conclusions of which have already been published (MacLeod, 1941). This work led to the evolution of a protective type of dip which is in fairly common use to-day in sheep districts where the tick is prevalent. In view of the present interest in new synthetic chemicals as possible constituents of tick dips, it is desirable that the protocols of this earlier work, and the experimental basis for the marketed 'anti-tick' dips, should be made known.

*Methods.* The field tests were made on hill farms in Argyll and Perthshire, on Blackface hoggs (tegs) from the ordinary stock on the grazing. In most cases eight to ten sheep were dipped in each preparation under test. An undipped control group was run with each experimental flock. The period of immersion was for $\frac{1}{2}$ or 1 min., the head being repeatedly submerged. The sheep were then allowed to graze at will on the tick-infested ground, and were examined every 3 or 4 days, or weekly. The degree of tick-infestation was assessed by counts of attached female ticks on the head, neck, shoulders and axillae (MacLeod, 1939).

All living female ticks, whether moribund or active, observed on the dipped sheep were counted, so that in many cases the infestations recorded give an unfair impression of the protection conferred, especially during the first few days after dipping, for many ticks recorded were newly attached and had not had time to die. This error was less important with coal-tar products, which usually kill within a few hours, than with arsenic, which may take over 24 hr. to kill. Derris may take 2 or even 3 days to kill ticks attached at the time of dipping, but this does not introduce so much error into the assessment of its protection against reinfestation as might be imagined, for unfed ticks, picked up by the sheep, are in many cases stupefied by the derris and so prevented from attaching. Unattached ticks on the wool or hair were not counted.

*Interpretation of results.* A working measure of the reliability of means based on groups of five and ten was calculated from a rather limited number of control counts. A thorough examination of this question has since been published by Milne (1943), and it is therefore unnecessary to give details here of the measure used beyond

comparing the values with the more accurate ones obtained by Milne from adequate data. Milne's values are in brackets:

| Mean | Difference for $P=0.05$ | |
|---|---|---|
| | Groups of 5 | Groups of 10 |
| 10 | — (8·07) | — (5·7) |
| *c.* 12 | 8·8 — | 6·2 — |
| *c.* 15 | 9·4 (11·28) | 6·7 (7·95) |
| *c.* 27 | 13·2 — | 9·3 — |
| 30 | — (20·82) | — (14·7) |

The period of time over which a dip continued to keep the adult infestation at less than 50% of the control is referred to as its 'effective duration'. This period does not represent the actual duration of protective effect. There is a 'lag' period of recovery of an infestation brought to zero by the actual dipping, so that, in the absence of any protective effect the infestation will take some days to equal once more that of the undipped controls. Under constant conditions of exposure to infestation, the period is, for female ticks, about 8 days, since the female usually feeds for 8 days. By plotting infestations as percentages of the control, the 'true protection area' may be delimited from the total area of protection in the figure by a curve of normal recovery based on a reinfestation rate of about 12% per day (Fig. 2).

## The effective duration of arsenic and coal-tar creosote

*Arsenic.* There is little recorded earlier work on the value of arsenic against *I. ricinus*; apart from the paper by Walton, already referred to, I know of only one reference—a description by Olenev & Pomerantzev (1928) of spraying tests on cattle against this species, in which they obtained a 64% kill in 39 hr. with a 0·2% solution of sodium arsenite.

The protection against reinfestation given by sodium arsenite, at concentrations equivalent to 0·2 and 0·16% $As_2O_3$, and by disodium thio-arsenate at 0·2% $As_2O_3$, is shown in Fig. 1*a*. This shows an effective duration of arsenic, for sheep, of about a fortnight, a period which contrasts markedly with that for cattle, estimated as 2 days (Graybill, 1913; Queensland Cattle Tick Committee, 1929) or 1 day (Legg & Foran, 1929).

This greater duration is probably related to the nature of the animal's coat in the regions infested by ticks. The female sheep tick attaches in marginal areas where short open wool intermingles with hair. That the presence of this wool should increase the retention period of the arsenic over that for purely hair-covered areas is suggested by the finding that in the wool-fleece proper soluble arsenic is retained, in amounts effective against blowfly larvae, for about 4 weeks (MacLeod, 1938; Hobson, 1940).

The individual tick counts for the three arsenical substances tested do not demonstrate that the one bath is more effective and more durable than the other.

If the two strengths of arsenite are compared directly, Fisher's '$t$' test for significance gives a probability of between 0·1 and 0·2 even on the 18th day, when the difference appears obviously significant by inspection. This is due to the scatter in the counts for the 0·16% bath group (range 1–29).

If, on the other hand, a general estimate of deviations of tick counts is used, the 18th day difference is significant; further, the thioarsenate becomes significantly less effective than arsenite of the same strength, on the 13th day reading.

*Coal-tar creosote.* Coal-tar creosote ('carbolic') dips consist of a mixture of cresols, hydrocarbons and tar bases, obtained at one stage of the distillation of coal tar. The two principal components, the tar oils plus bases and the phenols

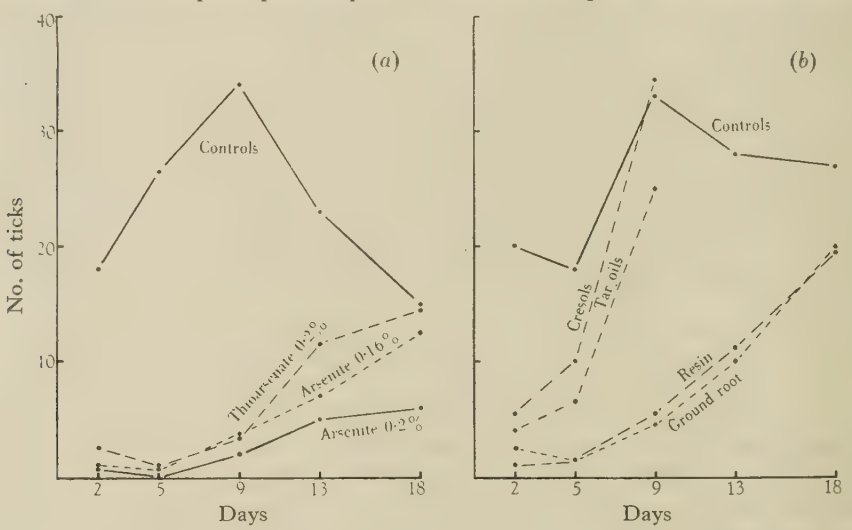


Fig. 1. Comparison of duration of toxicity (*a*) of arsenicals, (*b*) of cresols and of derris.

('cresylic acid'), were tested separately at bath strengths of 0·29% cresols and 0·76% tar oils. Observation of individual ticks showed that both dips were fairly efficacious in killing attached ticks, but, as shown in Fig. 2, they had little or no protective value against reinfestation.

*Combinations of arsenic and tar products.* For sheep dipping, carbolic dips have the advantage that they kill attached ticks more rapidly than does arsenic or derris. Further, their inclusion in other dips materially assists penetration through the fleece.

A test of a paste dip, containing sodium arsenite (0·2% $As_2O_3$) and coal-tar creosote (0·9% total tar products), gave the following results:

| Days after dipping | 5 | 8 | 11 | 15 | 19 |
|---|---|---|---|---|---|
| Average count: Dipped sheep | 1·2 | 1·6 | 2·5 | 4·3 | 3·1 |
| Controls | 15·7 | 12·5 | 8·1 | 8·8 | 4 |

i.e. an effective duration of about a fortnight.

In order to test whether a cumulative increase of protection occurs with repeated dipping, a group of fifty sheep were dipped five times at fortnightly intervals with a dip containing sodium arsenite (0·17% $As_2O_3$) and cresols (0·125%). For the third and subsequent dippings the strength was reduced by one-third to avoid damage to the sheep. The results showed no increase in protective value with successive dippings, through cumulative effect of the arsenic.

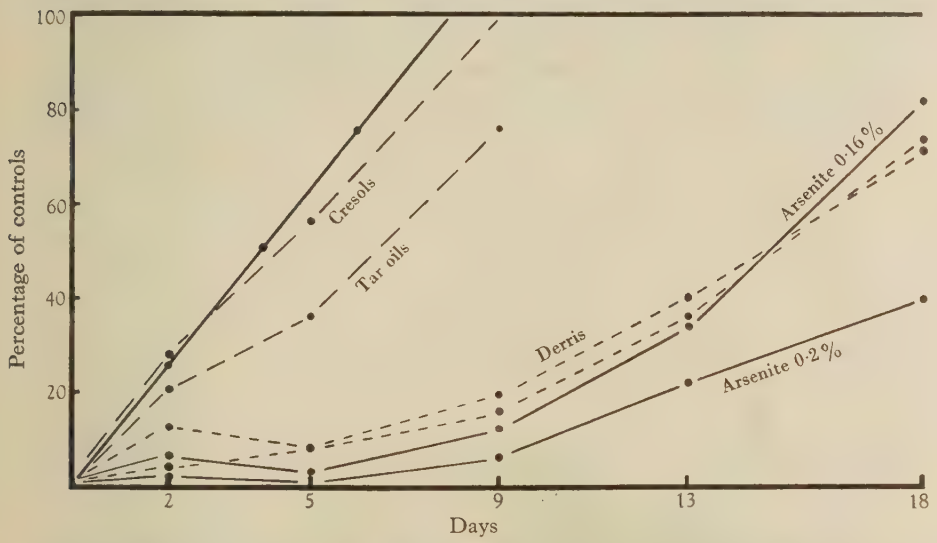


Fig. 2. Mean infestation expressed as percentage of control infestation. The straight line is the theoretical curve of recovery of an infestation where there is no protection. The derris curves are for extract and ground root, both at a concentration of 0·014% resin.

## The mode of action and effective duration of derris

The use of derris products against ticks, prior to this investigation, appears to have received practically no attention. The Queensland Cattle Tick Committee record, without details, that derris, though killing many ticks (*B. annulatus australis* Fuller) on cattle, was of little practical use in tick control.

In 1933, I recorded some preliminary experiments which indicated that derris root had a marked killing effect against *Ixodes ricinus*. The possibility of a protective effect was not then investigated, and, until the work of Milne (1945), the only information in the literature bearing on this question which I have been able to find is an observation by Clunies Ross (1935) that a water suspension of root is effective against *I. holocyclus* Neum on dogs and also protects the dogs against reinfestation for about 3 days.

The effect of derris on ticks has been studied both in the laboratory and field, and since no similar records have been published, I give my results in some detail.

*Laboratory tests.* For this work, a 10% solution of derris extract (27% rotenone) in diacetone alcohol was used. The solution included a small quantity of emulsifier, and dilutions were made in distilled water. Fig. 3 illustrates the rate of kill of unfed nymphs (groups of 24) immersed for 1 min. at a bath temperature of 20° C. in different concentrations of the extract. At a concentration of $10^{-3}$ or above, similar mortality rates were obtained with the extract emulsion and the corresponding concentrations of adjuvants (diacetone alcohol plus emulsifier) alone, but at lower concentrations the derris had clearly a specific effect (*b* and *c*).

Numerous other bench experiments have confirmed that derris extract emulsions in water are toxic at concentrations as low as $10^{-5}$, i.e. 1 part of extract in 100,000

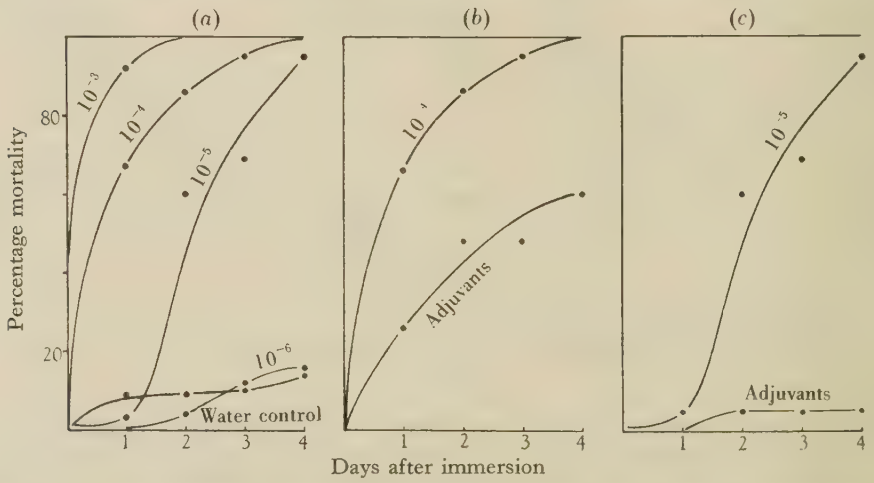


Fig. 3. (*a*) Mortality-time curves for nymphs immersed for 1 min. at 20° C. Concentrations expressed as extract to water. (*b*) and (*c*) Comparison of mortality curve with that for the solvent and emulsifier alone, at equivalent concentration.

of water. A constant feature of these tests was the slower onset of effect at the lower concentrations, as illustrated in the figure. This is also brought out in Fig. 4.

Tests with unfed larvae (Table 1) show that this stage also is highly susceptible to derris intoxication. The tests were made with the same derris preparation.

Both larvae and nymphs were killed when dusted with ground derris root.

*The mode of action of derris.* The susceptibility of the non-tracheate larval ticks to derris emulsions and dusts suggested that entry through the spiracles was not the only explanation of the action of this contact poison. The actual time when intoxication takes place was investigated by immersing two groups of twenty nymphs in a $10^{-4}$ concentration for 1 min. at 15° C. One group was then washed thoroughly under running water and dried lightly on blotting paper; the other was merely dried lightly to remove excess of the emulsion. The mortality curves are given in Fig. 4*a*

TABLE 1. *Susceptibility of tick larvae to derris emulsions*

Exp. 1: Result read after 6 hr.

| Extract + adjuvants | | |
|---|---|---|
| Dilution | No. of larvae | Mortality % |
| — | — | — |
| $10^{-2}$ | 50 | 100 |
| $10^{-3}$ | 65 | 100 |
| $10^{-4}$ | 71 | 98 |
| $10^{-5}$ | 59 | Not determined |
| $10^{-6}$ | 491 | exactly, many larvae alive |
| Water control | 54 | 24 |

Exp. 2: Result read after 5 days

| Extract + adjuvants | | | Adjuvants alone | | |
|---|---|---|---|---|---|
| Dilution | No. of larvae | Mortality % | Dilution | No. of larvae | Mortality % |
| — | — | — | $10^{-1}$ | 150 | 97 |
| — | — | — | $10^{-2}$ | 180 | 56 |
| $10^{-3}$ | 150 | 100 | $10^{-3}$ | 136 | 22 |
| $10^{-4}$ | 170 | 88 | $10^{-4}$ | 160 | 14 |
| $10^{-5}$ | 140 | 64 | $10^{-5}$ | 258 | 21 |
| $10^{-6}$ | 184 | 23 | | | |
| Water control | 210 larvae | | 21 % mortality | | |

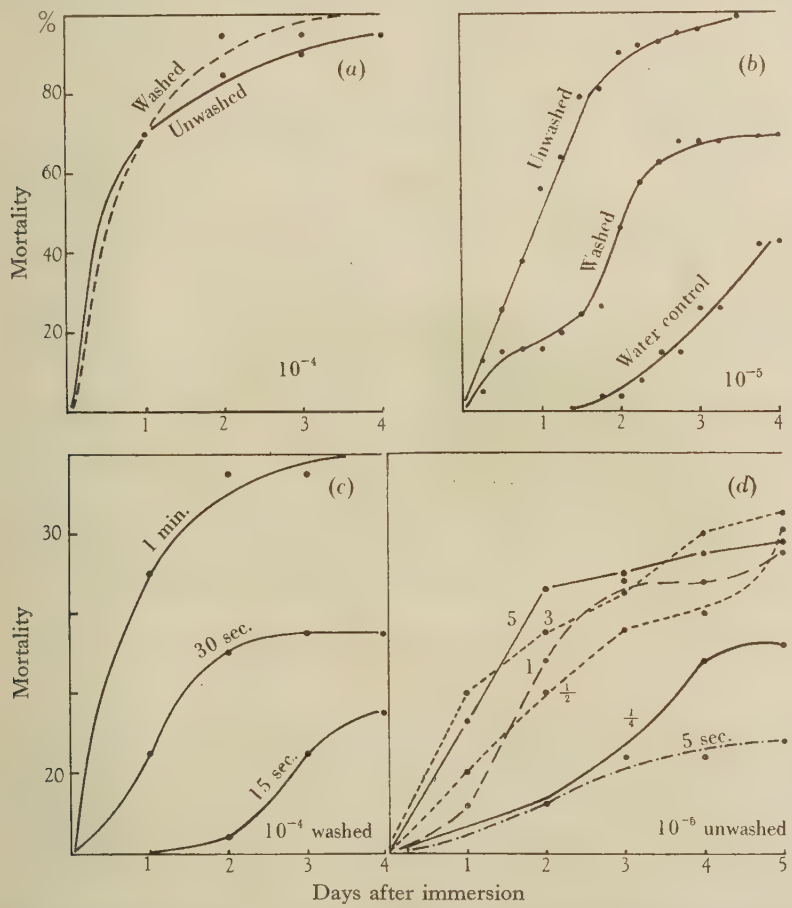


Fig. 4. (*a*) and (*b*) Effect of subsequent washing off of residual film of derris after 1 min. immersion at 15° C. in 1 : 10,000 and at 21° C. in 1 : 100,000 dilution of extract. (*c*) Effect of varying the immersion time, the nymphs being subsequently washed free of insecticide. (*d*) Comparison of different immersion periods, without subsequent washing.

and show no significant difference between the groups. This result, indicating that intoxication occurs during the actual immersion, was checked in another experiment, where observations were made at 6-hourly intervals. A lower concentration was used, and to check for the possible interfering effect of washing in a rather violent flow of water, the 'unwashed' group was subjected to a similar treatment before immersion in the derris emulsion. A control, washed in water, was included. Immersion was for 1 min. at 21° C. Fig. 4*b* illustrates the results.

This particular group of nymphs may have been more susceptible to intoxication than the others, for the 'unwashed' mortality curve is almost as high as those shown above for $10^{-4}$ concentrations; the bath temperature was, however, higher. The results show that, although the mortality is partially reduced by washing off the film of derris emulsion after immersion, there is a marked toxic effect occurring during actual immersion.

The above finding raises the question of the importance of immersion time, and some bench tests on this are illustrated in Fig. 4 (*c*, *d*). The (*c*) curves show the effect of immersion for 1, $\frac{1}{2}$ and $\frac{1}{4}$ min. in a $10^{-4}$ concentration, the groups of nymphs being subsequently washed free of surplus film of emulsion. The effect is comparable to that of varying the concentration, with constant immersion time, and further indicates that the residual film of derris emulsion is of minor importance compared to the actual contact of the wash during immersion. According to this test, immersion for $\frac{1}{2}$ min. is less effective than immersion for a full minute, but repeat tests, with the subsequent washing omitted (one of them is illustrated in Fig. 4*d*), showed that the minimum duration for full effect is not markedly above $\frac{1}{2}$ min.

*Reproducibility of results.* During the course of the laboratory work, which extended over several months, different groups of nymphs were used, which were bred out as required. There was a wide range of variation in the effect of given concentrations of derris extract on ticks from the different batches, so that it was frequently difficult to compare successive lots of results. Thus with ticks from ten batches, immersion for 1 min. at 20–22° C. in a $10^{-5}$ concentration of derris extract gave the following distributions of percentage mortality at 2 and 4 days:

| | 0–20% | 21–40% | 41–60% | 61–80% | 81–100% |
|---|---|---|---|---|---|
| After 2 days | 1 | 1 | 3 | 3 | 2 |
| After 4 days | — | 2 | — | 4 | 4 |

The possibility of the divergences being due to age differences in the batches of nymphs used was examined by simultaneous tests of nine different batches, of age varying from 5 to 42 days. The same degree of scatter was again obtained, but there was no relationship between age and susceptibility, and the variability must be assumed to be due to other differences in the ticks themselves, possibly related to the host on which they fed, or to the conditions under which the gorged larvae were maintained and moulted. It is clear that results obtained with ticks in the laboratory are at present only comparable when one group of ticks, of the same history, is used.

*Field tests.* Derris was tested for protective value in the field both as an emulsion of extract and as a suspension of ground root. Both baths were at a concentration of 1 part resin in 7000. The results are shown in Fig. 1 *b*. Derris, whether the toxic constituents have been isolated from or left in the root matrix, exercises an equal effect, and at the strength used is comparable in its effective duration to sodium arsenite at 0·16% $As_2O_3$ (Fig. 2).

A feature of the derris dip was the rapidity of its effect on unfed ticks. Repeatedly, in this and other experiments, it has been observed that many unfed ticks die before succeeding in attaching. Apparently, ticks picked up by the sheep, during the period of effective duration of the derris, are very often stupefied by the derris left on the fleece, and are unable to continue their search for a suitable attachment site; they

TABLE 2. *Effect of derris dipping on attached female ticks*

| Concentration | Sheep no. | No. of ticks originally attached | Living to dead ticks after dipping | | | Survival |
|---|---|---|---|---|---|---|
| | | | Day 1 | Day 2 | Day 3 | |
| 1:10,000 | 1 | 2 | | 0/2 | 0 | 0 in 36 |
| | 2 | 7 | | 0/6 | 0 | |
| | 3 | 27 | 12/15 | 0/14 | 0 | |
| 1:15,000 | 1 | 3 | 0/3 | 0/1* | 0 | 0 in 25 |
| | 2 | 9 | | 0/6 | 0 | |
| | 3 | 13 | | 0/15 | 0 | |
| 1:20,000 | 1 | 8 | 1/7 | 0/4* | 0 | 2 (1?) in 50 |
| | 2 | 16 | | 2/9 | 1† | |
| | 3 | 26 | | 2/23 | 1 | |
| 1:40,000 | 1 | 3 | 1/1 | 0/0* | 0 | 3 (2?) in 27 |
| | 2 | 5 | | 0/3 | 1‡ | |
| | 3 | 19 | | 2/13 | 2 | |
| 1:80,000 | 1 | 4 | 0/4 | 1/2* | 0 | 5 in 28 |
| | 2 | 10 | | 4/3 | 1 | |
| | 3 | 14 | | 4/4 | 4 | |

* The values marked thus indicate that the dead ticks have been dropping off.
† This tick, though still alive, was obviously affected.
‡ This may have been a newly attached tick.

remain clinging to the wool, or hair, until they die. This has not been observed with either arsenical or carbolic dips.

To test for the critical effective dilution under field conditions the infested sheep should, after dipping, be removed to non-infested pasture, to avoid confusion from reinfestation. This was not practicable, however, and sheep rather lightly infested, so that individual track of the ticks could be kept, were dipped, in groups of three, in concentrations of 1 part extract in 10,000, 15,000, 20,000, 40,000 and 80,000 of water. Moribund and dead ticks could not in all cases be distinguished at 24 hr. and only one sheep from each batch was examined. The results, after 24 hr. and 2 and 3 days, are given in Table 2, the ratio expressing the number of living to the number of dead female ticks of the original infestation.

The derris extract was effective at concentrations as low as 1 in 15,000, and killed a high proportion of attached ticks even at 1 in 80,000 or almost $10^{-5}$.

Table 3 gives the results of a test of the effect of (*a*) fouling, (*b*) standing for 1 week and (*c*) immersion time on the efficacy of derris baths. Two derris extract baths were prepared, at strengths of 1 in 5000. One was fouled by passing several sheep through it, and, in addition, faeces from the floor of the draining pen were added. A week later a fresh bath was prepared, and groups of ten sheep were passed through each, immersion being for $\frac{1}{2}$ min. A further group was immersed in the clean fresh bath for 15 sec. There is no significant difference between the kill or the effective duration of fresh or old, clean or fouled wash, with $\frac{1}{2}$ min. immersions; the '15 sec. immersion' group, on the other hand, shows a reduction in the kill and also a lessened protective effect. Complete saturation of the fleece of a blackface sheep

Table 3. *Effect of age and fouling of derris wash on efficacy against ticks*

| Age of dip | 1 week old | 1 week old | Fresh | Fresh | |
|---|---|---|---|---|---|
| Condition | Fouled | Clean | Clean | Clean | |
| Immersion time | 30 sec. | 30 sec. | 30 sec. | 15 sec. | |
| Days after dipping | | Tick counts (average of 10) | | | Undipped controls |
| 2 | 0·8 | 0·8 | 0·8 | 3·4 | 11 |
| 6 | 1 | 0 | 0·8 | 2 | 13·6 |
| 9 | 1·4 | 1·6 | 2·4 | 3·4 | 12·4 |
| 12 | 3·6 | 2·4 | 3·6 | 4·2 | 13·4 |

is achieved by less than 15 sec. immersion (judged by the cessation of air-bubble formation in the fleece), and in farming practice almost all dippings of hill flocks make use of intervals of from 10 to 20 sec. The effect of the lengthened immersion time is, therefore, interesting, and suggests that drainage from the areas occupied by the tick is very rapid, so that the ticks are in contact with free dipping fluid for only a few seconds after the sheep emerges from the bath. The effect demonstrated in laboratory tests is thus brought into operation, and so an actual immersion of at least 30 sec. is necessary in field practice.

An interesting demonstration of this was afforded by some dippings in Aberdeenshire. Serial dippings were being carried out in connexion with an eradication scheme, and were completely effective in killing attached ticks when the derris extract dip was used at a bath strength of 1 in 7000 of extract. As a result of the test with different concentrations of derris which indicated a threshold of complete efficacy below 1 in 15,000, I arranged to have the dip used at half strength, i.e. 1 in 14,000. The shepherds concerned reported that the dip was no longer killing ticks. I attended the next dipping, and found that the sheep were being passed through the bath at a rate which left each immersed for from 7 to 12 sec.!

### Experiments on prolonging the action of tick dips

Two methods of prolonging the effective duration of arsenic and of derris suggest themselves, namely, increasing the strength, and incorporating them in a greasy or oily vehicle which will tend to make them adhere for longer periods to the wool, hair, or skin. Wool grease, as a natural constituent of the sheep's skin and fleece, seemed the most obvious vehicle. Emulsions were prepared of (*a*) unsaponified wool grease, (*b*) a mixture of saponified wool grease, plus the unsaponifiable fraction, and (*c*) mineral oil emulsified with a small amount of wool grease. These were tested first by themselves for possible protective effect—a not unlikely possibility, since greasy-skinned ewes are frequently less heavily infested than dry-skinned grazing under the same conditions.

The emulsions were diluted to bath strengths of 0·75% wool grease or mineral oil. Eight sheep were dipped in each and eight controls were run with them. Counts were made after 2, 5 and 9 days, and gave the following averages:

| Days | Wool grease | Saponified wool grease | Mineral oil | Controls |
|---|---|---|---|---|
| 2 | 17·5 | 10 | 18·5 | 20 |
| 5 | 18·5 | 18 | 25·5 | 17 |
| 9 | 41 | 30 | 35 | 33 |

The relatively low count for the saponified wool grease on the 2nd day suggests that this wash had some degree of killing effect, probably due to the action of the soaps. None of the baths gave any protection against reinfestation.

*Carbolic.* A test with coal-tar creosote incorporated in wool grease was made. The results were:

4th day Infestation 45% of controls
11th day Infestation 100% of controls

Since the normal recovery rate (12% per day) would achieve only about 50% by the 4th day, it is clear that the wool grease had not enhanced the effective duration of this dip.

In another test a combination of cresols and saponified wool grease was tried, with a control of the wool grease alone. The results were:

| Days after dipping | 7 | 14 | 20 | 23 | 28 | 32 |
|---|---|---|---|---|---|---|
| Undipped control | — | 12·9 | 19 | 25·2 | 20·1 | 23·3 |
| Wool grease | 5·5 | 9 | 13·8 | 16·4 | 16·4 | 17·2 |
| Wool grease plus cresols | 5·5 | 11·1 | 16·4 | 16·8 | 16·7 | 21 |

The persistent though slight reduction of infestation may indicate some effect, but this, if a true one, must obviously be due to the wool grease. It is, in any case, negligible (see Fig. 5*b*).

*Arsenic.* Some preliminary tests were made with arsenic and wool grease, but attention was principally concentrated on the effect of adding wool grease to

a combination of arsenic and cresols, for, in the absence of the antiseptic action of the cresol, the wool grease was found rapidly to turn rancid.

Sodium arsenite and cresol were incorporated with saponified wool grease to give a bath strength of 0·17% $As_2O_3$ and 0·65% wool grease. The average tick counts, compared with those of controls dipped in a bath of the same arsenical strength but without wool grease, are shown in Fig. 5*a*. The groups were of only six sheep, so that the significance of the differences on the 11th, 14th and 17th days is lower than would be expected from inspection; the values, determined by '*t*' test, i.e. even ignoring the effect of a third group, are:

| | 11th day | 14th day | 17th day |
|---|---|---|---|
| *P*= | 0·08 | 0·07 | Just over 0·4 |

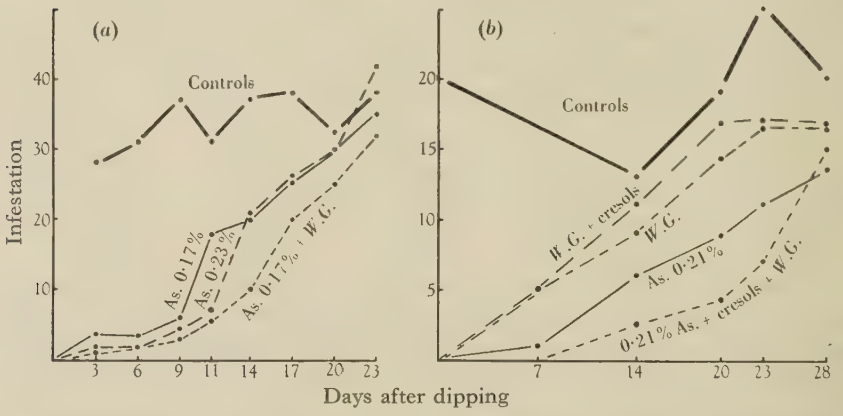


Fig. 5. (*a*) Effect on protection of increasing the arsenical concentration and of adding wool grease (*W.G.*). (*b*) Effect of adding wool grease to cresols, and to an arsenic-cresol combination.

An extra group was tested with the 'arsenite plus cresols' bath increased from 0·17 to 0·23%. It is interesting that a greater prolongation effect was obtained by adding wool grease than by increasing the arsenical strength.

A similar dip to the above wool-grease dip, but with the arsenical concentration increased to 0·23% $As_2O_3$, gave a greater period of partial protection. The infestations, expressed as percentages of the controls, for comparison with the above results, were as follows:

*Arsenite plus wool grease bath*

| Days after dipping | 6 | 9 | 11 | 17 | 20 | 21 |
|---|---|---|---|---|---|---|
| Average count (%) (0·17% $As_2O_3$) | 6·5 | 8 | 27 | 53 | 85 | — |
| Average count (%) (0·23% $As_2O_3$) | 3 | 5·5 | 24 | 40 | — | 46 |

The effect of using total tar products instead of the separated phenolic fraction was tested by comparing two dips, the arsenic and wool-fat strength of which were

kept equal (0·21% $As_2O_3$, 0·38% wool grease). No significant difference in the protection was obtained, and the result confirms the previous finding that 'carbolic' plays no part in the actual protection conferred by arsenic and carbolic combinations. The results (average tick counts) were:

| Days after dipping | 7 | 14 | 20 | 23 | 28 | 32 |
|---|---|---|---|---|---|---|
| Arsenic + wool grease + coal-tar creosote | 0·3 | 1·9 | 4·3 | 6·6 | 13·9 | 20 |
| Arsenic + wool grease + cresols | 0 | 2·5 | 4·3 | 7·1 | 15·1 | 16·3 |
| Undipped controls | 3·1 | 12·9 | 19 | 25·2 | 20·1 | 23·3 |

Fig. 5*b* gives the details of an experiment where an arsenic-cresols-wool-grease dip was compared with its individual components. The arsenical strength in this case was 0·21% ($As_2O_3$).

Groups of eight sheep were dipped in each bath. The arsenic bath was not strictly comparable, as sodium arsenite was used in the compound dip, whereas the arsenic bath was made from a powder dip containing a mixture of arsenical substances partly in trivalent and partly in pentavalent form. The bath strength was equivalent to 0·2% $As_2O_3$. The effective duration of the arsenic alone is difficult to

TABLE 4. *Concentration of derris wash and duration of protection.*

| | Mean infestation per sheep | | | | | |
|---|---|---|---|---|---|---|
| Days after dipping | 1/1000 | 1/2000 | 1/5000 | 1/10,000 | 1/15,000 | Controls |
| 3 | 0·1 | 0·1 | 0·6 | 0·8 | 2·7 | 14·3 |
| 7 | 0·9 | 1·8 | 5·2 | 10·6 | 16·8 | 41·8 |
| 10 | 1·8 | 2·9 | 9·8 | 20·5 | 20·4 | 46·7 |
| 14 | 5·9 | 8·9 | 18·9 | 26·5 | 22·6 | 43 |
| 17 | 12·7 | 10·4 | 22·5 | 24·9 | 24·3 | 34·6 |
| 21 | 14·8 | 17·2 | 34 | 21·8 | 25·3 | 25·5 |

determine in this case, since, although the protection dropped to about 50% by the end of a fortnight, it remained at approximately that level until the 23rd day. The combination of arsenic, cresols and wool grease continued to give a high degree of protection for 23 days, at which time the infestation was only 28% of the control. The differences between the 'arsenic plus wool grease' and the 'arsenic' group are statistically of low significance. Even if the experiment is treated as a straight comparison between the two, the other groups being ignored, the '*t*' test gives levels of significance of just over 0·08 on the 14th day, and only under 0·2 on the 20th day.

*Derris.* Since derris is innocuous to mammals when applied to the outer skin, the concentration of the wash can be increased indefinitely, the limiting consideration being purely one of cost. Derris extract at 1 in 1000 was taken as the maximum concentration from the point of view of commercial practicability. Dilutions ranging from this to 1 in 15,000 were tested, groups of ten sheep being used for each. The sheep were clean when dipped, having newly returned from wintering on a lowland farm.

Table 4 gives the detailed results, which are illustrated in Fig. 6 as percentage of the controls.

The curves for 1 in 15,000 and 1 in 10,000 strength show little difference, and indicate a rather low protective value for derris at such concentrations; the 1 in 5000 strength gave a fairly high degree of protection for 10 days, after which the effect rapidly disappeared. The two baths at increased strength, 1 in 1000 and 1 in 2000, gave identical and almost complete protection for 10 days and a marked degree of protection for 14–17 days.

The results from the 7th to 17th day have been examined for significance by analysis of the variance. The control group has been omitted except in the last (17th day) test, since it is so obviously different from the others that its inclusion would obscure the test. The '$Z$' value is on each occasion far beyond its 1% point, indicating a highly significant association between the group mean and dipping strength.

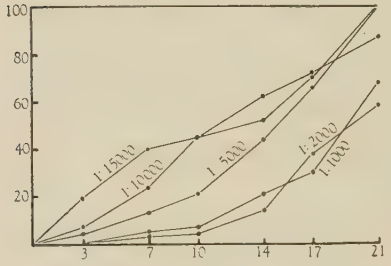


Fig. 6

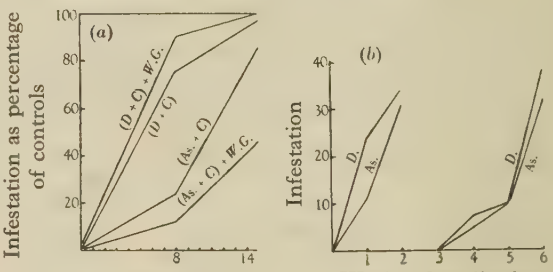


Fig. 7

Fig. 6. Protection curves, as percentage of controls, for different concentrations of derris extract. Note the close correspondence of the two highest concentrations.

Fig. 7. (*a*) The effect of adding wool grease to derris (*D*) and to arsenic. Tar products (*C*) were used with both dips. (*b*) The effect of hurried immersion on protection (weeks 1 and 2) compared with the results after proper dipping (weeks 3–6).

The grouping into categories, significantly different from each other, of the six lots, is as follows:

| | | | | |
|---|---|---|---|---|
| 7th day | 1 in 1,000 | 1 in 10,000 | 1 in 15,000 | Controls |
| | 1 in 2,000 | | | |
| | 1 in 5,000 | | | |
| 10th day | 1 in 1,000 | 1 in 5,000 | 1 in 10,000 | Controls |
| | 1 in 2,000 | | 1 in 15,000 | |
| 14th day | 1 in 1,000 | 1 in 5,000 | 1 in 10,000 | Controls |
| | 1 in 2,000 | | 1 in 15,000 | |
| 17th day | 1 in 1,000 | | 1 in 5,000 | Controls |
| | 1 in 2,000 | | 1 in 10,000 | |
| | | | 1 in 15,000 | |

It will be observed that at no time did the two highest strengths differ significantly in the intensity of protection; it is probable that the duration of the protection period (14–17 days) represents the maximum effective duration of derris extract washes on areas liable to tick infestation, i.e. areas exposed to light.

Ground derris root incorporated in a coal-tar creosote emulsion, to give a bath strength of 1 in 7000 (0·014%) derris extract, and 0·76% tar products was tested with and without saponified wool grease (0·5%). In a repeat test, the above derris root + carbolic + wool-grease dip was compared at a strength equivalent to 1 in 5000 extract, against a derris extract bath of this strength and an arsenic powder bath at 0·2% $As_2O_3$. In both tests the degree of infestation of the pastures was found to be disappointingly low, but the results (Table 5) are sufficiently clear to show that the addition of wool grease did not enhance the effective duration of the derris. In the second test the effect is apparently rather better with than without the wool grease, but since a ground root + carbolic combination is compared with an extract emulsion, the conclusion is unsafe. Neither derris wash had a longer effective duration than that of arsenic at 0·2%, so that obviously no prolongation of effect occurred.

TABLE 5. *Effect of wool grease on duration of efficacy of derris against ticks*

| | Derris concentration 1 in 7000 extract | | | Derris concentration 1 in 5000 extract | | | |
|---|---|---|---|---|---|---|---|
| Day | Derris root + carbolic | Derris root + carbolic + wool grease | Controls | Derris resin | Derris root + carbolic + wool grease | Arsenic 0·2% $As_2O_3$ | Controls |
| 5 | 1·1 | 1·5 | 15·7 | 0·7 | 0·7 | 0·4 | 8·9 |
| 8 | 2·1 | 2·2 | 13·4 | 1·25 | 0·9 | 1·15 | 7·0 |
| 11 | 3 | 3·2 | 8·1 | 3·7 | 1·0 | 1·4 | 6·2 |
| 15 | 5·2 | 4·8 | 8·4 | 3·6 | 2·9 | 1·75 | 4·6 |
| 19 | 4·4 | 4·1 | 4 | | | | |

A field test on a large scale was made with baths of derris root at a strength equivalent to 1 in 7000 extract, plus tar products, with and without saponified wool grease. Sodium arsenite (0·2% $As_2O_3$), with and without wool grease, was also tested; in the former case with coal-tar creosote as an adjuvant, in the latter with cresols. This last dip (sodium arsenite plus cresols plus wool grease) also contained derris (1 in 7000), and is in fact the dual purpose tick-protective dip finally evolved (see Discussion). This test was carried out in collaboration with Mr W. Lyle Stewart, Newcastle University, who has published a brief account of the results (Lyle Stewart, 1939). The tick counts (Table 6) confirm that the presence of wool grease prolongs the effective duration of arsenic, but not of derris.

TABLE 6. *Effect of wool grease on duration of efficacy of derris and of arsenic*

| Days after dipping | Derris carbolic | | Derris carbolic + wool grease | | Arsenic carbolic | | Arsenic cresylic + derris and wool grease | |
|---|---|---|---|---|---|---|---|---|
| | Dipped | Controls | Dipped | Controls | Dipped | Controls | Dipped | Controls |
| 8 | 39·3 | 52 | 23 | 25·9 | 5·5 | 23·7 | 4 | 30·5 |
| 15 | 72·8 | 74·9 | 65 | 40·1 | 31·8 | 37 | 23 | 50·8 |
| 21 | — | — | — | — | 14·7 | 16·4 | 18·3 | 14·5 |

When the curves are expressed as percentage infestations, for purposes of comparison (Fig. 7*a*) they illustrate a rather important point. The dippings were done

in this case by shepherds, and it was subsequently verified that their customary rate of dipping allowed the sheep an immersion period of no more than 15 sec. The protection from both the arsenic and the derris is markedly reduced in comparison with that which I obtained (approximately a fortnight), but the reduction is far more striking in the case of the derris. That this reduction is related to the insufficient immersion time was corroborated by another field experiment, also in collaboration with Lyle Stewart. A breeding flock was dipped, by the shepherds themselves and without supervision by us, half in an arsenic powder dip (wash strength 0·2% $As_2O_3$) and half in a derris-root dip (0·037% extract). Counts after 1 and 2 weeks made it obvious that the protection, especially by the derris, was negligible. The dipping was repeated, by me personally, the sheep getting a ½ min. immersion. The bath was found to be improperly calibrated, so that the actual washes, at the first dipping, were only five-sixths the calculated strength. The correct strengths were used for the second dipping. The infestations after the two dippings are compared in Fig. 7*b*. After the second dipping the counts remained low for a fortnight, and it is clear that the inferior results in the first dipping could not be due to the reduction in strength alone (0·17% instead of 0·2% arsenic and 0·03% instead of 0·37% derris). Obviously the immersion time in the first dipping was insufficient, and the greater effect of this on the derris than on the arsenic is noteworthy.

Incidentally, the relatively poor result at the second dipping, for the rather high concentration of derris used, was in all probability due to the very uneven distribution of the derris root, which in this particular dip was observed to settle out rapidly in the bath.

## Discussion

Wool grease failed to increase the duration of effect of derris, even when the derris was in the form of ground root, the adherence of which to the hair and skin might reasonably be expected to be increased by the greasy vehicle. This suggests that the breakdown of protection is determined not so much by loss of the derris, through leaching or such cause, as by chemical change of the active constituents, under stress of weather conditions, and especially of sunshine. This is supported by the finding that increase of concentration from 0·05 to 0·1% of extract, in an emulsion bath, failed to increase the effective duration beyond 14–17 days, and it seems that this represents the maximum period for which derris remains active, when distributed in a thin film, at almost body temperature, and under the influence of light.

There is some evidence, on the other hand, that the effective duration of arsenic can be prolonged, by addition of wool grease, from about a fortnight (for a strength of 0·2% $As_2O_3$) to about 3 weeks. Based on these findings a protective dip was evolved, containing arsenic, derris, cresols and wool grease. Some results with this dip have been given by Milne (1945). Because of the incorporation of cresols, it is a dual-purpose tick dip, since it rapidly kills those ticks attached at the time of dipping as well as appreciably lowering the degree of subsequent infestation of the sheep for a period of about 3 weeks. During the first week or 10 days of this period,

most of the ticks coming on to the sheep are being killed by the derris before they succeed in attaching; the sheep are thus to some extent being protected from infection with disease agents. After the derris effect becomes negligible, the protective role devolves on the arsenic, which continues, for the remainder of the protection period, to kill many of the ticks which attach.

The following is a typical field result obtained with this dip:

| Days after dipping | 11 | 14 | 18 | 21 |
|---|---|---|---|---|
| Control count: (average of 10) | 12·5 | 20 | 23 | 12·5 |
| Percentage reduction in dipped group | 80 | 82 | 70 | 17 |

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(*Received* 19 *October* 1946)

# THE FORMATION OF INSECTICIDAL FILMS ON BUILDING MATERIALS

## II. TESTS OF THE EFFICIENCY OF VARIOUS TYPES OF PRETREATMENT

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(With 1 Text-figure)

Thirty different pretreatments have been tested, in addition to those mentioned in a previous contribution, for their efficiency in prolonging the effective toxic life of a film of pyrethrins in Shell Oil P31 deposited on brick. Reasons for the success or failure of the various types of pretreatment are considered. Seven substances selected for further trial were tested on four more building materials—limewashed brick, wood, cement, and cement-sand mixture. The five pretreatments which gave good results on all or most of these substrates were then tested to determine how long they would continue to support a toxic film on cement. Solutions of size and gelatin caused the greatest persistence of toxicity of the pyrethrum films. The flour beetle, *Tribolium castaneum* Herbst, was used throughout as the test insect.

### Introduction

Parkin & Hewlett (1947) showed that films of pyrethrum or D.D.T. in Shell Oil P31 deposited on certain common building materials rapidly lost their toxicity to the flour beetle, *Tribolium castaneum* Herbst. They also recorded preliminary tests which showed that the persistence of toxicity could be greatly increased by pretreating the surface of such materials with a suitable substance, e.g. starch paste, before application of the insecticide. In this paper an account is given of the results of tests with a wide range of substances to select those most effective as pretreatments.

### Materials and methods

Unless otherwise stated, the samples of building materials and the technique of spraying with insecticide were similar to those employed previously. The test insects were adult *Tribolium castaneum* Herbst, bred and conditioned by the method described by Parkin & Green (1943). The insects were 3–5 weeks old when first confined on films, except in the experiments on the relation between toxicity and film age. The insecticide employed throughout was 1·6% w/v pyrethrins in Shell Oil P31, each solution being used on the day on which it was made up. Storage of the experimental samples and exposure of insects on the treated surfaces were carried out at 25% C. and 70% R.H.

All pretreatments were applied with a brush, and, with two exceptions, as a single coat. Paints, resins and varnish-like substances were allowed to dry for at least 48 hr., and the linseed oils for 11 days. All aqueous solutions and suspensions were left to dry for at least 16 hr.

Every effort was made to keep the amount of pretreatment applied to the samples constant, and tests indicated that the variation between the amounts applied to different samples was probably not more than $\pm 25\%$, which was not considered excessive in view of the nature of the investigation. More accurate methods were discarded, because of inherent difficulties which did not seem capable of quick solution.

## Preliminary tests of a number of materials as pretreatments

### *Experimental technique*

Two improvements were made to the technique employing portions of Fletton bricks as the substrate for pretreatment (see Parkin & Hewlett, 1947). First, the samples were dried by baking for 12 hr. in an oven at approximately 95° C., so that their moisture content was probably uniformly low (1% or less), and comparable to that found on the inside walls of most buildings. Dried bricks do not absorb moisture at relative humidities below about 70%, so the samples were stored indoors and used as required. Secondly, samples used for later tests were vacuum-cleaned, because it was suspected that brick dust on the surface increased the toxicity of the pyrethrum film formed; and since the dustiness of the samples appeared to be variable, this was considered an important contributory cause of the heterogeneity of some earlier results. The increase in toxicity due to dust was confirmed experimentally, and the mortality observed on non-pretreated, vacuum-cleaned bricks used in later tests of pretreatments was, in general, lower than before the introduction of vacuum cleaning.

### *Pretreatments*

As it was hoped to discover a pretreatment suitable for practical use on a large scale, attention was concentrated on substances commercially available. A list of substances for test was drawn up by the authors and, in addition, a brief statement of the problem was sent to a number of commercial firms, with a request for samples of preparations they thought worthy of trial.

Proprietary products supplied ready for use were applied undiluted, except the phenol-formaldehyde resin, the high viscosity of which necessitated dilution with absolute alcohol. The concentrations of the solids applied as viscous dispersions in water were adjusted so that the viscosities of the dispersions were very approximately the same.

The material described as 'proprietary sodium silicate' is a preparation sold commercially for sealing the pores in brickwork, etc., and was applied undiluted; those described as 'dilute proprietary sodium silicate' and 'dilute waterglass (egg preserver)' were applied to the samples in two coats, the first and second coats being respectively 17 and 20% v/v solutions in water. The magnesium sulphate-calcium chloride wash consisted of a thin creamy suspension of calcium sulphate in aqueous magnesium chloride (sp. gr. 1·18). A full list of pretreatments is given in Table 2.

*Results*

Each pretreatment was placed in one of the classes shown below, according to whether the persistence of the toxic film was increased, decreased, or unaffected.

| Class of pretreatment | Persistence of film |
|---|---|
| A | Markedly increased |
| B | Slightly increased |
| C | Unaffected |
| D | Slightly decreased |
| E | Markedly decreased |
| AE | Markedly increased in some replicates, markedly decreased in others |

Table 1 shows the quantitative definition of the designations given to the different pretreatments. A very few isolated replicates gave figures outside the ranges of knock-down and mortality given in this table, but these could safely be ignored, especially when the 9-day as well as the 6-day counts were taken into consideration. The designation D, included above for the sake of completeness, could not be assigned to any of the pretreatments tested after the preliminary experiments (see Parkin & Hewlett, 1947) and so is not included in Table 1.

Table 1. *Description of pretreatment classes by ranges of knock-down and mortality obtained in batches of fifty adult* Tribolium castaneum, *exposed 6 days on films formed by 3·90–4·08 mg./sq.cm.* of 1·6% total pyrethrins in P31; films 24 hr. old when exposure began*

| Bricks not pretreated | | | | Bricks pretreated | | |
|---|---|---|---|---|---|---|
| Knock-down % | Mortality % | Mortality % in control† | Class of pretreatment | Knock-down % | Mortality % | Mortality % in control† |
| 90–100 | 2–26 | 0–14 | A | 100 | 90–100 | 0–4 |
| 90–100 | 10–38 | 0–4 | B | 98–100 | 6–92 | 0–8 |
| 90–100 | 6–26 | 0–14 | C | 80–100 | 2–16 | 0–4 |
| 100 | 24–35 | 6 | E | 4–20 | 0–16 | 0–4 |
| 90–100 | 2–25 | 0–10 | AE | 78–100 or 0–42 | 66–100 or 0–42 | 0–10 |

* The range of deposits was narrower in individual tests.
† I.e. surfaces not sprayed.

In Table 2 the pretreatments are listed, and grouped in their classes.

The pretreatments tested in the preliminary experiments (see Parkin & Hewlett, 1947) can also be graded according to the classification given in Table 1. Size, polyvinyl alcohol, undiluted waterglass, and starch paste would be designated as A, calcium chloride solution and water (light application) as C, and water (heavy application) as D.

It was decided to select for further work pretreatments which (1) were placed in class A and (2) showed no obvious practical disadvantages. Such practical difficulties were unsuitable colour (one of the enamels), very high viscosity at effective concentrations (sodium silicate and waterglass), and recommendations for use as more

than one coat (both enamels). The pretreatments so selected included all three polyvinyl acetal emulsions and both grades of sodium alginate. One of the emulsions was chosen arbitrarily, and one grade of sodium alginate because it adhered to brick better than the other. The pretreatments finally selected were therefore 10% w/v size, 5% w/v polyvinyl alcohol, starch paste, 5% w/v gelatin, 2½% w/v sodium alginate, polyvinyl acetal emulsion, and brickwork sealing paint.

TABLE 2. *Pretreatments arranged according to their efficacy on brick*

Class A

Saturated aqueous sodium silicate
Proprietary sodium silicate
Enamels (two types)
Polyvinyl acetal emulsions (three types)
Brickwork sealing paint
5% w/v gelatin (edible grade) in water
2½% w/v sodium alginate (two grades) in water

Class B

Canada balsam in xylol (dilute)
7% w/v soluble starch in water
Red oxide paint

Class C

Dilute proprietary sodium silicate
Dilute waterglass (egg preserver)
Magnesium chloride—calcium sulphate wash

Class C (*cont.*)

Urea-formaldehyde resin
2% w/v methyl cellulose in water
1% w/v chondrus jelly

Class E

Raw linseed oil
Pale boiled linseed oil
Ethyl cellulose emulsion

Class AE

Cellulose acetate in amyl acetate
Bakelite varnish
Linseed oil-based paints (three types)
Phenol-formaldehyde resin
Polyvinyl chloride emulsion

## FURTHER INVESTIGATIONS OF THE SEVEN SELECTED PRETREATMENTS

### *Toxicity of films on four building materials pretreated with the seven selected substances*

The seven substances selected in the experiments described above were tested to discover whether they were effective as pretreatments on surfaces other than brick. The toxicity of pyrethrum films on limewashed brick, wood, cement and cement-sand treated with each of the seven pretreatments was compared with that on the corresponding non-pretreated surfaces; starch paste on limewashed brick was not tested, because, in drying, the paste stripped the limewash off the bricks.

*Experimental technique.* The deposit of pyrethrins in P31 was kept at approximately 4 mg./sq.cm., except on wood. The deposit on wood was lowered to about 2 mg./sq.cm., for 4 mg./sq.cm. on this material would have been too high to obtain a comparison. For each pretreatment and material there were two replicates: with cement and cement-sand the free* surface of one sample was sprayed, and the moulded* surface of the other. Fifty insects were put on to each surface 24 hr. after spraying, and the numbers of beetles knocked down and dead counted 6 and 9 days later.

*Results.* The data obtained are summarized in Table 3. Each pretreated surface was classed as + or o, defined as follows:

+ Mortality greatly increased
o Mortality not, or only slightly, increased

} when compared with the mortality on the corresponding surface not pretreated.

* For the meaning of these terms, see Parkin & Hewlett (1947).

Table 3. *The effect of pretreatment upon pyrethrum films formed on different building materials. For the definition of '+' and 'o' see text*

| Pretreatment | Surface | | | | | | |
|---|---|---|---|---|---|---|---|
| | Brick* | Lime-washed brick | Wood | Cement (free) | Cement (moulded) | Cement-sand (free) | Cement-sand (moulded) |
| Size | + | o | + | + | + | + | o |
| Polyvinyl alcohol | + | o | o | o | o | + | o |
| Sodium alginate | + | o | o | o | o | o | o |
| Brickwork sealing paint | + | + | o | + | + | + | + |
| Polyvinyl acetal emulsion | + | + | o | + | + | + | + |
| Starch paste | + | | + | + | + | + | + |
| Gelatin | + | + | + | + | + | o | o |

* This column was derived from earlier experiments on brick.

If knock-down had been the criterion, polyvinyl alcohol on cement (free) and on cement-sand (free), and sodium alginate on cement-sand (moulded), would have been rated +. In the case of sodium alginate on wood, the toxicity of the film was decreased by the pretreatment; but a − category was not introduced, since it was not possible to tell whether pretreatment of cement and cement-sand decreased the toxicity of the insecticidal film, because of the low toxicity of the films formed on these substrates (see Parkin & Hewlett, 1947).

No further work was carried out using polyvinyl alcohol and sodium alginate, because they were effective upon very few of the surfaces.

In most of the experiments described above heavy deposits of insecticide of about 4 mg./sq.cm. were employed. The deposit in practice, however, is much less, viz. about 1·0–1·5 mg./sq.cm., so the effect of lowering the deposit on pretreated surfaces was investigated. Samples of cement (free surface) pretreated with 10% w/v size, brickwork sealing paint, polyvinyl acetal emulsion, starch paste, and 5% w/v gelatin, were sprayed with 1·6% w/v pyrethrins at a deposit of 1·5 mg./sq.cm. Insects were confined on the surfaces 24 hr. later, and counts were made after the insects had been exposed to the films for 6 days.

On most of the samples 100% mortality was obtained—in no case less than 94%; the mortality with a 4 mg./sq.cm. deposit on non-pretreated cement was nil. In view of these results a deposit of 1·5 mg./sq.cm. was employed in subsequent laboratory experiments.

*The relation between film toxicity and film age on pretreated cement*

Experiments were carried out to determine the rate of decline of toxicity of pyrethrum films on cement (free) pretreated with the five materials chosen on the basis of previous work, viz. 10% w/v size, brickwork sealing paint, polyvinyl acetal emulsion, starch paste, and 5% w/v gelatin (edible grade).

*Experimental technique.* In testing each pretreatment, a series of pretreated samples was sprayed on the same day with approximately 1·5 mg./sq.cm. of

insecticide, and batches of insects were confined on the surfaces at different times after spraying. The knock-down and mortality of the insects were counted after 3, 6, and 9 days exposure on the films. The insects for each set of pretreated samples were taken from one subculture containing beetles 3–5 weeks old at the start of the experiment, so that they were not all of the same age when exposed. This procedure was adopted because an opportune time for exposing a batch of insects could be

TABLE 4. *The relation between film toxicity and film age on pretreated cement. The figures in the body of the table are percentage mortalities obtained from batches of fifty insects. Deposit:* 1·40–1·54 *mg./sq.cm.*

| Pretreatment | Exposed on films for (days) | Initial film age (days) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Size | 3 | 100 | 90 | 100 | — | 58 | — | 34 | — |
| | 6 | 100 | 100 | 100 | — | 94 | — | 92 | — |
| | 9 | 100 | 100 | 100 | — | 100 | — | 100 | — |
| Brickwork sealing paint | 3 | 98 | 50 | 2 | 22 | 8 | 0 | 2 | 2 |
| | 6 | 98 | 68 | 2 | 28 | 8 | 2 | 2 | 4 |
| | 9 | 100 | 80 | 6 | 44 | 18 | 4 | 2 | 6 |
| Polyvinyl acetal emulsion | 3 | 100 | 100 | — | 92 | — | — | 74 | — |
| | 6 | 100 | 100 | — | 100 | — | — | 94 | — |
| | 9 | 100 | 100 | — | 100 | — | — | 96 | — |
| Starch paste | 3 | 96 | 58 | — | 8 | — | 26 | — | 27 |
| | 6 | 100 | 94 | — | 24 | — | 88 | — | 85 |
| | 9 | 100 | 100 | — | 30 | — | 100 | — | 94 |
| Gelatin | 3 | 34 | 62 | — | 56 | — | 46 | — | 22 |
| | 6 | 94 | 98 | — | 98 | — | 88 | — | 86 |
| | 9 | 98 | 100 | — | 100 | — | 100 | — | 100 |

| | | Initial film age (days) 9 | 10 | 11 | 13 | 14 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|
| Size | 3 | 56 | — | — | — | — | — | 28 | — |
| | 6 | 94 | — | — | — | — | — | 84 | — |
| | 9 | 100 | — | — | — | — | — | 100 | — |
| Brickwork sealing paint | 3 | — | — | — | — | — | — | — | — |
| | 6 | — | — | — | — | — | — | — | — |
| | 9 | — | — | — | — | — | — | — | — |
| Polyvinyl acetal emulsion | 3 | 62 | — | 20 | — | 2 | — | — | 4 |
| | 6 | 86 | — | 38 | — | 18 | — | — | 8 |
| | 9 | 92 | — | 76 | — | 54 | — | — | 10 |
| Starch paste | 3 | — | — | 36 | — | 16 | 14 | — | — |
| | 6 | — | — | 88 | — | 30 | 36 | — | — |
| | 9 | — | — | 100 | — | 30 | 36 | — | — |
| Gelatin | 3 | — | 25 | — | 25 | — | — | — | 6 |
| | 6 | — | 71 | — | 76 | — | — | — | 32 |
| | 9 | — | 88 | — | 86 | — | — | — | — |

judged, to some extent, from the mortality among those of the series preceding it. Moreover, it was known that the resistance of *T. castaneum* to pyrethrum under laboratory conditions increases after emergence until an age of 3–5 weeks is reached, and remains approximately constant for several weeks thereafter.

*Results.* The results are given in Table 4.

In Fig. 1 the initial age of the film has been plotted against the percentage mortality of the insects after 6 days exposure. The figure shows that the pretreatments may be placed in descending order according to the durability of the films formed upon them—size, gelatin, starch paste, polyvinyl acetal emulsion, and brickwork sealing paint.

In the further interpretation of Table 4 and Fig. 1, it should be remembered that the toxicity of a film was falling during the confinement of a batch of insects upon

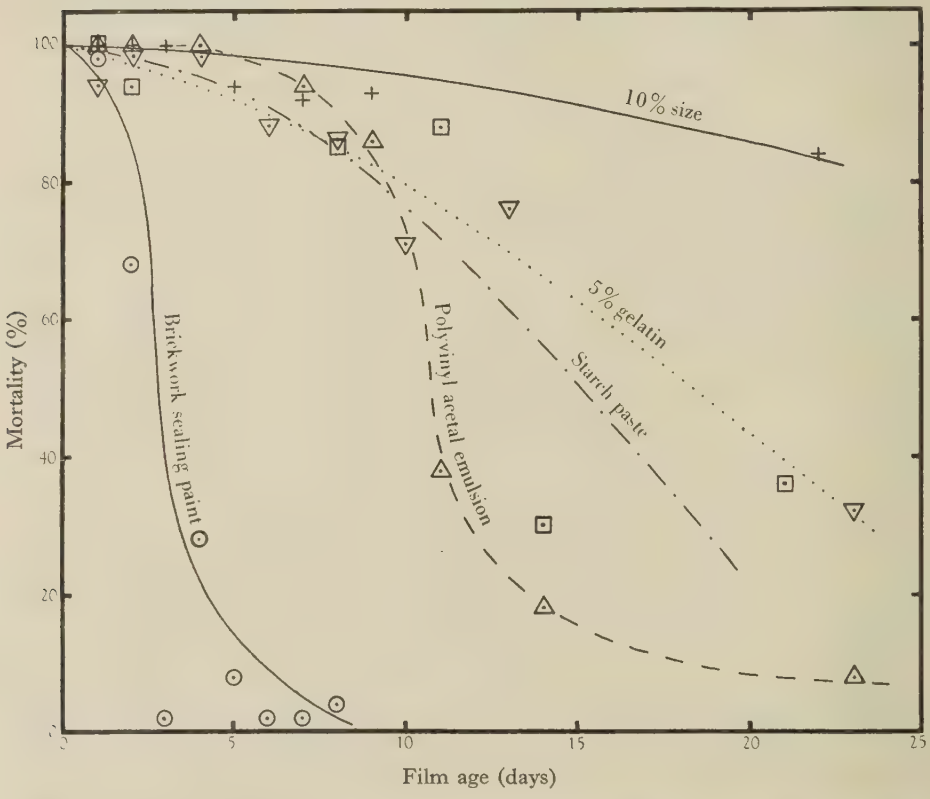


Fig. 1. Curves showing the persistence of toxicity of pyrethrum films on cement pretreated with different substances. Ordinate: percentage mortality after exposure of insects on films for 6 days. Abscissa: age of film at start of exposure of insects.

it, so that the figure for mortality represents an average film toxicity during the corresponding exposure period. Evidence of this was obtained on brickwork sealing paint, when exposure of batches of insects began respectively 4 and 5 days after deposition of the films. The percentage knock-down was initially 100, but fell during the exposure period.

Table 4 shows that, for a given 9-day mortality less than 100%, the difference between 3-day and 9-day mortalities varies considerably according to the pretreatment concerned. In general, the extent of the difference follows the order given above for the permanence of the films. The magnitude of this difference between 3-day and 9-day mortalities reflects the rate of loss of toxicity of the film; for the more rapidly a film is losing its toxicity during the exposure of a batch of insects upon it, the more slowly will the mortality rise towards the end of the exposure period.

The data plotted in Fig. 1 fit sigmoid or incomplete sigmoid curves reasonably well. Dosage-mortality curves are generally accepted to be sigmoid (Clark, 1933), dosage being some measure of the amount of poison available to act on the organism. The sigmoid shape of the curves in Fig. 1 therefore indicates that there is some simple relation, e.g. linear or logarithmic, between the availability of insecticide on a pretreated surface and film age.

## Discussion

Parkin & Hewlett (1947) discussed the relative toxicities of pyrethrum in P31 films in relation to (*a*) the volume of liquid insecticide transferred from the substrate to the cuticle of the insects and (*b*) the concentration of active principle in (*a*). As (*a*) undoubtedly depends on the amount of insecticide left on the surface, the effectiveness of the pretreatments will be discussed in relation to this factor.

An insecticidal film consists initially of free insecticide (pre-absorption film) and later of insecticide left on the surface after capillary absorption is complete (post-absorption film). In all experiments the films on non-pretreated surfaces reached the post-absorption stage well before the insects were exposed on them. In the tests of pretreatments on brick, the change from pre-absorption to post-absorption films on pretreated surfaces occurred before, during, or after exposure of the insects, according to the pretreatment. In these tests it was observed that where the insects were exposed to a pre-absorption film during all their 6-day exposure period the pretreatment showed as A; if all the exposure period was spent on a post-absorption film the pretreatment might show an A, B, C or E result; while if partly on a pre-absorption and partly on a post-absorption film the result was A or B.

The AE results require special interpretation. It was observed that the A replicates invariably occurred where the pretreatment formed a more dense coating over the surface of the brick. The nature of the coating almost certainly depended upon the absorptive properties of the particular sample. When insects were exposed on a pre-absorption film for any of the exposure period, toxicity was high, but, when entirely on a post-absorption film, toxicity was either high or low. This, taken in conjunction with a tendency for mortality to equal knock-down, probably means that films on the AE pretreatments were of high toxicity initially, but the toxicity decreased relatively rapidly until it was less than that of the post-absorption film of brick not pretreated.

The linseed oils, ethyl cellulose, and brickwork sealing paint were the only pretreatments sufficiently oleophobic to cause the film of insecticide to contract into separate lenses, though in all these cases the contact angles were considerably less than 90°. The linseed oils and ethyl cellulose emulsion were classed as E, while brickwork sealing paint, though classed as A in the tests on brick, was shown in later tests to support films of only short duration.

Where the pretreatment coating, e.g. polyvinyl acetal and gelatin, was transparent and relatively impermeable to the insecticide, it was observed that insecticide was lost through macroscopic fissures in the pretreatment coating, caused for example, by incomplete coverage of cracks. The substrate was discoloured under the pretreatment coating along each side of a fissure. If fissures, macroscopic or microscopic, cause an important loss of insecticide from insecticidal films on pretreated surfaces, the results mentioned in the preceding paragraph are understandable. On a pretreatment coating not wetted by insecticide, the insecticide might be absorbed through the fissures (provided that the contact angle was less than 90°), leaving no insecticide on the surface; if the coating was wetted, similar absorption might take place, but a film of insecticide would be left on the surface.

From the foregoing discussion it appears that a substance will be most effective as a pretreatment if it forms a coating which is (1) relatively impermeable to the insecticide and (2) wetted by the insecticide.

On consideration of the results of the work described, it was decided to investigate size and gelatin further with a view to their employment as pretreatments in practice, and an account of these investigations will be given later.

We gratefully acknowledge the co-operation of numerous commercial firms in providing samples of their products for test; and the help given by Dr D. G. R. Bonnell and his colleagues of the Building Research Station in a discussion on building materials and possible pretreatments. The work has formed part of the programme of research of the Pest Infestation Laboratory and this account is published with the permission of the Department of Scientific and Industrial Research.

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(*Received* 21 *October* 1946)

# A DIPPING APPARATUS FOR ESTIMATING THE TOXICITY OF INSECTICIDES IN LIQUID MEDIA

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(With Plate 4 and 3 Text-figures)

An apparatus and a technique are described for the study of the effect of liquid insecticidal preparations by dipping insects in the toxic material. The method is applicable to quick-settling suspensions and quick-creaming emulsions as well as to solutions. Dipping is performed at constant temperature with end-over-end shaking; and the technique has the advantage over the other dipping methods that have been described in that handling of individual insects whilst wet is eliminated.

## Introduction

Various laboratory methods of evaluating the toxicity of contact insecticides in liquid medium have been described, and, if techniques adapted for special purposes, e.g. the evaluation of fly sprays, are excluded, they fall into two classes:

(*a*) Spraying of insects placed on a solid surface (Tattersfield & Morris, 1924; Potter, 1941; Jones, Campbell & Sullivan, 1935).

(*b*) Immersion of the insects in the toxic fluid (Kearns & Martin, 1935; Fryer, Stenton, Tattersfield & Roach, 1923; Fleming & Baker, 1934; Shepard & Richardson, 1931; Craufurd-Benson, 1938).

Spraying methods have been widely used but have their disadvantages, some of which have been enumerated by Craufurd-Benson (1938). One of the difficulties encountered in a spraying tower is the problem of obtaining an evenly distributed insecticide deposit of constant weight over the whole area on which the insects are confined. Special precautions must therefore be taken to ensure that the spraying apparatus is kept in a place free from draughts; and the whole apparatus, particularly the atomizer, must be carefully constructed, as slight variations or defects in its construction can lead to serious irregularities in the deposit obtained. The same problem of distribution arises in the construction of a dusting apparatus.

There is considerable evidence to show that, in order to obtain reproducible results, the temperature and humidity conditions affecting the insects both before and after, but notably after, spraying, must be carefully controlled (Potter & Gillham, 1946). The temperature at which the spraying is actually carried out is another factor in determining the kill. A constant temperature and humidity room in which to house the spraying apparatus is therefore desirable. In using a dipping technique the toxic material can be kept at constant temperature is a thermostatically controlled

water-bath. All that is otherwise necessary is a constant temperature-constant humidity cabinet in which to keep the insects before and after treatment.

A number of dipping techniques have been worked out. Kearns & Martin (1935) dipped eggs of permanent apple aphis and apple sucker on twigs by immersing them for 15 sec. in emulsions of organic rhodanates. Fryer *et al.* (1923) dipped caterpillars for 10 sec. in derris extracts. No great accuracy was claimed for this method. Fleming & Baker (1934) immersed Japanese beetles in alkali oleate solutions for 120 sec., and Shepard & Richardson (1931) dipped *Aphis rumicis* in nicotine solutions for 15 min. Finally, Craufurd-Benson (1938) described a technique for obtaining accurately reproducible results by dipping *Ahasverus advena* in a suspension of derris resin in water for 4 min. under carefully controlled conditions.

None of these methods, however, would appear to be satisfactory when the dipping fluid is a suspension in which the particles have an appreciable rate of sedimentation. The apparatus here described was originally built to test suspensions of this type; and it can be used for coarse or quick-creaming emulsions also, as well as for solutions or finely divided emulsions or suspensions, when the question of sedimentation during the period of dipping does not arise. The insects are immersed in the liquid contained in a small tube, closed at both ends, and subjected to end-over-end shaking under the surface of the water of a constant-temperature bath. The technique has the further advantage over other methods in that handling of individual insects whilst still wet with dipping fluid is entirely eliminated.

## TEST INSECTS

The insects used in this apparatus, so far, have been adult *Tribolium castaneum* Hb., *Oryzaephilus surinamensis* L. and *Ahasverus advena* Waltl., but any insects of comparable size should be suitable.

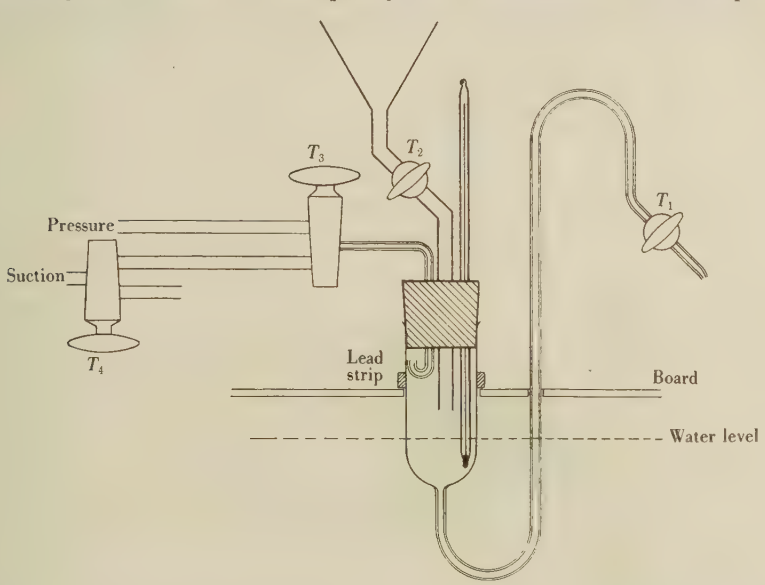
## DESCRIPTION OF APPARATUS

A water-bath ($10 \times 8 \times 18$ in.) is kept at a constant temperature, using a 'Sunvic' thermostat and relay operating a 1 kW. electric immersion heater. The underwater stirrer-shaker assembly is constructed mainly of Meccano parts. A $\frac{1}{50}$ h.p. Edward's motor drives the (horizontal) stirrer, using chain drives; and the same motor also operates the end-over-end shaker, consisting of a clip (*C*, Pl. 4, fig. 1), attached to a Meccano wheel, and situated about $1\frac{1}{2}$ in. below the surface of the water of the constant-temperature bath. This clip holds the dipping tube (see below). The shaker can be stopped and started by means of a worm-wheel clutch arrangement (*A* and *B*, Pl. 4, fig. 1) without disturbing the continuously running stirrer. The shaker turns at about 20 r.p.m. As the chain drives pick up and scatter water over the apparatus, it is advisable to put metal shields over some of the moving parts (*J*, Pl. 4, fig. 1).

The dipping tubes are 2 in. lengths of 1 in. diameter glass tubing. When in use they are closed at either end with a rubber stopper, kept in place by a short length

(about $\frac{1}{2}$ in.) of 1 in. diameter rubber tubing (*J*, Pl. 4, fig. 2). These rings serve a double purpose. Without them the stoppers tend to come off under water; and they also prevent mixing of water from the bath with the toxic material in the tube.

When not is use, the insecticide material is contained in a reservoir made from a boiling tube to the bottom of which is sealed a lead of capillary glass tubing (Text-fig. 1). This is used when the suspension is transferred from the reservoir to the dipping tube, and is fitted with the tap $T_1$. The stopper of the reservoir carries a thermometer, a delivery tube connected to an ordinary 7 cm. filter funnel by a tap $T_2$, and a capillary tube connected to the system of two-way taps $T_3$ and $T_4$. It is advantageous if the end of this capillary tube is doubled back to a U-shape below




Text-fig. 1. Fluid system (not to scale).

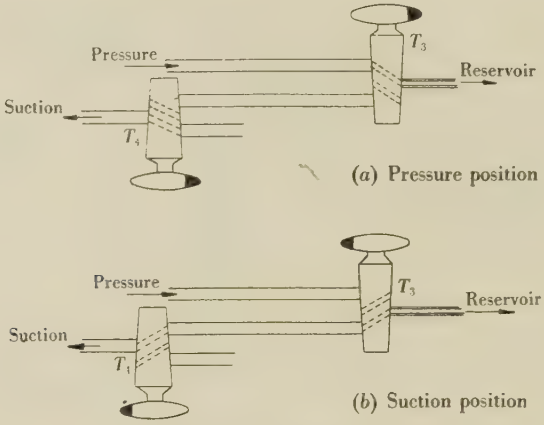
the stopper of the reservoir, since, in the process of sucking back the fluid from the dipping tube into the reservoir through the funnel, splashing may occur. The U-bend prevents the suspension from accidentally being drawn into the tube leading to the taps $T_3$ and $T_4$. The free tubes leading from $T_3$ and $T_4$ are connected to the blowing and suction leads respectively of a filter pump air blower.

The reservoir, funnel, taps, heater and heat control are mounted on an asbestos board on top of the bath. It is convenient also to have two spare boiling tubes mounted on this board so that fresh suspensions can be heated up while another is in use (*D*, Pl. 4, fig. 2). These are kept in position by winding round the middle of each a strip of lead sheet held in place by insulating tape. This serves to weight the tube and acts as a rim keeping the tube firmly in its hole in the board. This method is also

used to keep the reservoir in position. The funnel and taps $T_1$ and $T_2$ are held in place by clamps attached to a small retort stand bolted to the board. A small light dipping below the water surface is also useful. In this case a 6 V. motor-car bulb is used, operated through a transformer from the a.c. mains. It is contained in a 100 ml. tall beaker resting in a hole in the board (*H*, Pl. 4, fig. 2). Taps $T_3$ and $T_4$ are wired to the board.

*Operation*

(1) *Manipulation of taps.* By adjusting $T_3$ and $T_4$ to position (*a*) (Text-fig. 2), air pressure is applied to the reservoir, and by this means the toxic material is driven from the reservoir into the dipping tube. With $T_3$ and $T_4$ in position (*b*) suction is applied, and this is used to drain off the insecticide from the insects.




Text-fig. 2. Manipulation of taps.

(2) *Duration of dipping.* In the technique as described below, a period of dipping of less than 30 or 40 sec. is impracticable; a longer period of about 10 min., although not impracticable, is not suitable in view of the desirability of carrying out tests as rapidly as possible. A period of 2½ or 3 min. is therefore recommended. Experiments showed that there are no apparent ill effects, if the above species of insects are dipped for this period in the water-acetone-wetter mixture, used extensively in this laboratory as a medium in the preparation of suspensions of solid insecticides. The time necessary to produce drowning will of course vary from species to species. Craufurd-Benson reports that *Ahasverus advena* has been immersed in water for an hour without drowning; and Shepard & Richardson have immersed *Aphis rumicis* for an hour in distilled water and found them unaffected.

(3) *Treatment of insects.* A standard amount (usually about 8 ml.) of insecticide material is introduced into the reservoir through the funnel and $T_2$ with $T_3$ and $T_4$ in position (*b*), and is allowed to attain the temperature of the bath, normally about

25° C., $T_2$ being closed, and $T_3$ and $T_4$ changed to position (*a*). One would not expect the volume of liquid used to be of critical importance. The amount of air, however, entrapped with the insects and fluid may be so. It is therefore advisable to standardize the volume of material used. No more material is introduced into the reservoir than is actually used in the dipping tubes. This ensures that, when using suspensions, concentrated sediments are not drawn off from the bottom of a larger bulk of material. Ten or fifteen insects are placed in a dipping tube, with the stopper and ring in position at one end. As $T_3$ and $T_4$ are now in position (*a*), the fluid is blown into the dipping tube by simply opening $T_1$. Simultaneously a stop-watch is started. The open end of the tube is now quickly sealed with a second stopper and ring, and the tube placed in the clip of the shaker which is at once caused to rotate by means of the clutch. With a little practice, this operation, from the time of opening $T_1$ till the tube is placed under water in the shaker, can be performed in 10 sec. The loss of temperature involved is about 0·5–1° C., and this is quickly regained by the tube in the shaker.

Towards the end of the dipping period, $T_3$ and $T_4$ are set to position (*b*) and $T_2$ is opened; about 15 sec. before the end, the shaker is stopped and the tube withdrawn. One ring and stopper are removed and are replaced by a piece of muslin (about 2 in. square) kept in place by a rubber band.

At the instant of completion of the dipping period, the suspension is poured through the muslin back into the reservoir by way of the funnel, the remaining stopper and ring being simultaneously removed from the other end of the tube. In this way the bulk of the liquid is drained from the insects. Further drying is effected by pressing the end of the tube carrying the wet muslin on to pads of filter paper. The open end can now be closed with a second piece of dry muslin; and the tube is kept, dry end down, on filter paper at constant temperature for 24 or 48 hr. before inspection of the insects. When the insects dry, the majority of them fall down on to the dry muslin.

The rubber stoppers are dried on filter paper and are ready for the next tube. The reservoir and connected tubes can easily be washed out at any stage by conducting some washing fluid (e.g. acetone) through the system in exactly the same manner as has been described for the toxic material.

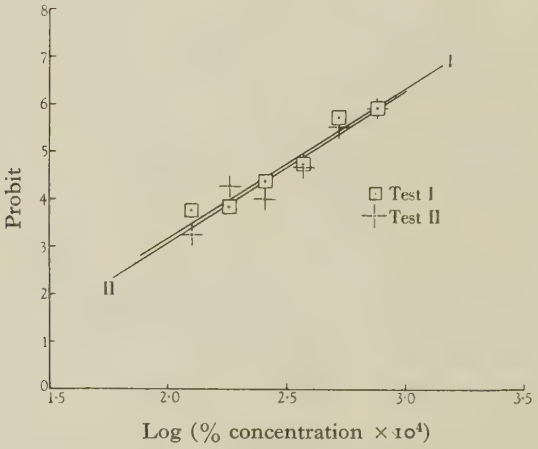
### *Performance*

No data are yet available for the performance of the apparatus in comparison with any spraying or other testing technique; but provided normal precautions are taken, the results are, for D.D.T. preparations at least (the only compound tested so far), consistent and reproducible. Dosage-mortality curves can be transformed to straight lines by standard methods of probit analysis. The two lines shown below, for example, are replicates obtained for the same D.D.T. preparation tested on the same day against the same culture of *Tribolium castaneum*. Each line is the calculated

'best fit' for the corresponding six points. There is obviously very little discrepancy between the two. Deaths in the controls are very infrequent.

TABLE 1

| % concentration of D.D.T. | Log (conc. × $10^4$) | % kill | Probit |
|---|---|---|---|
| | Test 1 | | |
| 0·075 | 2·88 | 82 | 5·92 |
| 0·053 | 2·72 | 76 | 5·71 |
| 0·037 | 2·57 | 40 | 4·75 |
| 0·026 | 2·41 | 27 | 4·39 |
| 0·018 | 2·26 | 12 | 3·83 |
| 0·0126 | 2·10 | 11 | 3·77 |
| Slope and standard error: | | | 3·13 ±0·37. |
| Log (median lethal conc. × $10^4$) and standard error: | | | 2.585±0·029. |
| | Test 2 | | |
| 0·075 | 2·88 | 82 | 5·92 |
| 0·053 | 2·72 | 70 | 5·52 |
| 0·037 | 2·57 | 38 | 4·69 |
| 0·026 | 2·41 | 16 | 4·01 |
| 0·018 | 2·26 | 24 | 4·29 |
| 0·0126 | 2·10 | 4 | 3·25 |
| Slope and standard error: | | | 3·19±0·37. |
| Log (median lethal conc. × $10^4$) and standard error: | | | 2·610±0·029. |




Text-fig. 3. Duplicate tests by dipping technique.

## DISCUSSION

The most obvious disadvantage of the method is the time required to treat each batch of insects in comparison with the time taken (about 15 sec.) for a similar operation in a spraying tower.

It is possible that the effect of dipping an insect in the manner described above

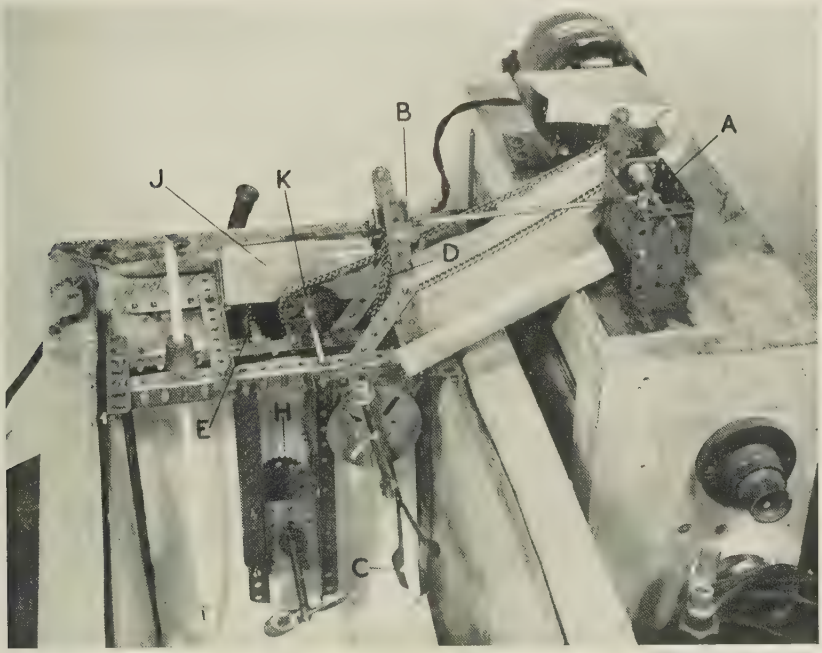



Fig. 1

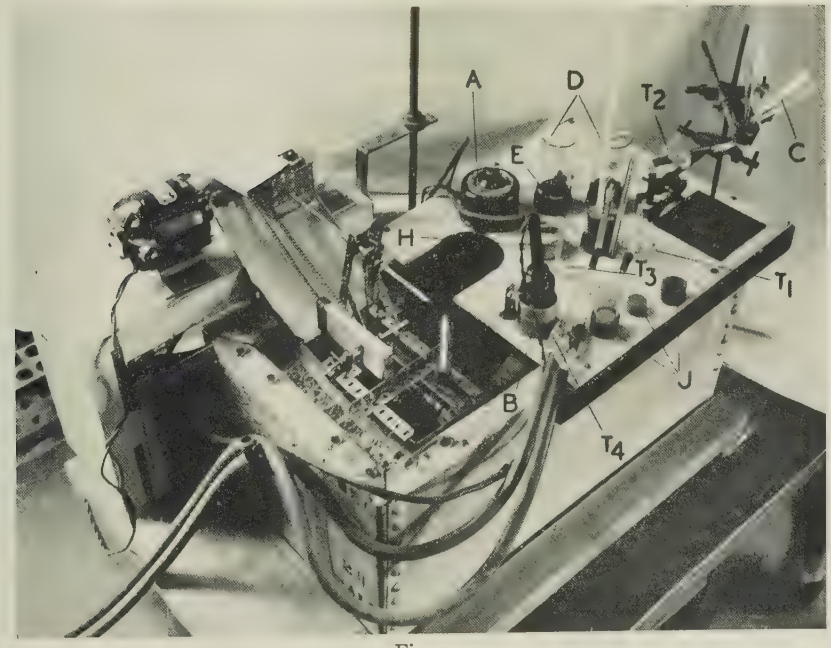



Fig. 2

may not be comparable with the effect of spraying as practised in the field. A greater penetration into the tracheae may be produced by immersion than by spraying, and the immersed insects may swallow the dipping fluid, thus producing the complication of a stomach poison effect. Until it can be established, therefore, whether insects do or do not absorb liquid in the way suggested, it would perhaps be hardly justifiable to apply results, obtained from dipping tests only, to developing spray mixtures for field uses. This, however, should not detract from the value of the dipping technique as a means of conducting strictly comparative laboratory trials, as it has a number of distinct advantages over any spraying method. It can be used for any type of fluid insecticide (solution, emulsion, or suspension), and it obviates the need for control of several rather variable factors which arise in a spraying tower, such as the degree of atomization, the rate of settling of droplets, and the extent of cooling and electrical charging of the droplets as a result of atomization, all of which affect the total weight and evenness of the deposit; and the temperature of treatment can more easily be controlled in a dipping than in a spraying technique.

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## EXPLANATION OF PLATE 4

Fig. 1. *A* and *B*, worm-wheel clutch arrangement. As shown it is in the 'engaged' position; to disengage, the lever *B* is raised and the long shaft withdrawn about $\frac{1}{2}$ in. towards the left, disengaging the cog from the worm wheel at *A*. The lever is in the 'disengaged' position in Fig. 2. *C*, clip of end-over-end shaker. This is attached to the large Meccano wheel and is turned by a sprocket wheel on the long shaft by means of the chain, *D*. The shaft carrying the stirring paddle (at bottom) also carries the sprocket wheel *H*, and is turned by the large sprocket wheel (under cover *J*) by means of the chain *E*. The motor drives the wheel *K*, which is on the same shaft as the large sprocket wheel operating *E*.

Fig. 2. *A*, 'Sunvic' heat control device; *B*, electric immersion heater; *C*, 7 cm. funnel—see Text-fig. 1; *D*, spare reservoirs; *E*, top of reservoir shown in Text-fig. 1; *H*, cork carrying 6 V. lamp; *J*, dipping tube, stoppered at one end, together with second stopper and ring; $T_1$, $T_2$, $T_3$ and $T_4$, taps—see Text-figs. 1 and 2.

(*Received* 29 *June* 1946)

# CALCIUM CHLOROACETATE AS A SOIL DRESSING AGAINST BEET EELWORM, *HETERODERA SCHACHTII* SCHMIDT, WITH CERTAIN ADDITIONAL OBSERVATIONS

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Calcium chloroacetate at 3 and 6 cwt./acre produced significant increases in the yields of sugar beet (both washed beet and total sugar) in a trial on fen soil of the 'skirt' type infested with beet eelworm, *Heterodera schachtii* Schmidt. However, the material had no effect on the eelworm content of the soil whether measured by cysts, viable cysts or eggs and larvae. An instance is recorded of a significant drop in the level of the beet eelworm infestation in the presence of sugar beet.

## 1. Introduction

Smedley (1938), using a laboratory technique, investigated the potentialities of certain chloroacetates as soil dressings for use against the potato-root eelworm, *Heterodera rostochiensis* Woll. These substances were shown to have a toxic effect on the larvae and cysts, and at least one of them, viz. ammonium chloroacetate, was considered to have practical possibilities. Field tests with calcium chloroacetate were carried out by O'Brien, Gemmell, Prentice & Wylie (1939) and Edwards (1939), who obtained an increased yield of ware potatoes without, however, reducing the level of the eelworm population.

In 1938, and again in 1939, the writer carried out field tests with the same substance for use against beet eelworm, *H. schachtii* Schmidt. The material was obtained from Imperial Chemical Industries under the reference number A27. The results were communicated in the confidential reports of the Sugar Beet Research and Education Committee of the Ministry of Agriculture and Fisheries; they are now being published in summarized form, first to supplement the findings of O'Brien *et al.* and Edwards, and secondly to put on record certain observations on the complex interaction between beet eelworm and the sugar-beet crop.

## 2. Preliminary trial, 1938

A preliminary trial was carried out in 1938 on a 'beet-sick' field in Fordham Fen, Hilgay, Norfolk. The soil was of the fen type known as 'light skirtland'—a light peaty loam. A strip along one edge of the field was divided into fourteen plots. These were dug on 16 and 17 March, after calcium chloroacetate had been broadcast at the rate of 4 cwt./acre on alternate plots. The sugar-beet seed was sown 3 weeks later. No differential effect on germination was noticed nor was there any difference in plant population on 18 June, on which date cysts were found on the roots of specimen plants from all plots. By 15 July, however, there was a very marked difference in the appearance of the tops, those on each treated plot being larger and

greener than those on the neighbouring untreated plots. Symptoms of manganese deficiency were much more pronounced on the untreated plots than on the treated plots.

On 6–8 October the beets were lifted and topped. The tops were weighed on the field and the roots sent to the British Sugar Corporation beet-sugar factory at Stoke Ferry, King's Lynn, where the yields of washed beet and the sugar contents were assessed.

TABLE 1. 1938 *trial: effect of calcium chloroacetate on yields*

| | Washed beet (tons/acre) | Sugar content (%) | Total sugar (cwt./acre) | Tops (tons/acre) |
|---|---|---|---|---|
| Treated | 6·07 | 13·7 | 16·6 | 1·51 |
| Untreated | 4·49 | 13·2 | 11·9 | 1·25 |

### 3. FURTHER TRIAL, 1939

In 1939 the test was repeated at Chatteris, Isle of Ely. The soil was again of the fen 'skirt' type but with a considerable admixture of clay. A simple randomized block arrangement was adopted with four treatments replicated six times:

(1) Control; no calcium chloroacetate, no carbonate of lime.

(2) Carbonate of lime at a rate equivalent in calcium to calcium chloroacetate at 6 cwt./acre.

(3) Calcium chloroacetate at 3 cwt./acre.

(4) Calcium chloroacetate at 6 cwt./acre.

Soil samples for eelworm estimations were collected on 22 March. The chloroacetate was applied to the appropriate plots on 23 March and immediately raked in. The carbonate of lime was applied on 29 March and a complete fertilizer to the whole trial area on 12 April. The seed was drilled on 13 April.

Owing to the rather poor tilth on the experimental area, the low seed rate employed and the dry weather, germination was rather irregular. During singling it was observed that a pigmy mangold beetle attack had occurred and that 'stringy root' (a pathological condition attributed to adverse soil conditions) was also quite common. These troubles were not observed to be in any way associated with the treatments. After singling, the plants on all plots made good growth and compared favourably with crops in neighbouring fields where the eelworm infestation was lower. Bolting was negligible on all plots.

The beet was lifted and topped during the period 26–29 September and soil samples were taken on 3 October. The tops were weighed on the field and the roots were dealt with at the Peterborough beet-sugar factory. In addition to the yield of washed beet and the sugar content, the 'number of beets' was also recorded. It is important to note that this quantity was obtained by counting the beets left on a standard washer which retained only those roots with a diameter greater than $\frac{3}{4}$ in.

The methods used for extracting the cysts from the soil samples and the estimation of the eelworm infestation in terms of cysts, viable cysts and eggs and larvae have been described by Jones (1945).

## 4. RESULTS

The data from the 1938 trial, though not suitable for statistical analysis, gave a strong indication of increased yield of washed beet, total sugar and tops as the result of using chloroacetate at the rate of 4 cwt./acre (Table 1).

The following summary of results refers to the 1939 trial (Tables 2 and 3). For convenience, the following symbols are used for treatments: $O$ = control, $L$ = carbonate of lime, $X$ = single dose of chloroacetate, $2X$ = double dose.

TABLE 2. 1939 *trial: effect of calcium chloroacetate on yields*

| | Control | Carbonate of lime | Calcium chloroacetate at 3 cwt./acre | Calcium chloroacetate at 6 cwt./acre | Mean | S.E. |
|---|---|---|---|---|---|---|
| *Beets per acre | 28,286 | 29,511 | 31,227 | 31,416 | 30,115 | 464·8 |
| Percentage of mean | 93·9 | 98·0 | 103·7 | 104·3 | 100 | 1·54 |
| *Washed beet (tons/acre) | 11·94 | 12·29 | 13·22 | 13·65 | 12·78 | 0·182 |
| Percentage of mean | 93·48 | 96·21 | 103·48 | 106·82 | 100 | 1·42 |
| Mean wt. of single beet (lb.) | 0·946 | 0·934 | 0·951 | 0·973 | 0·951 | 0·018 |
| Percentage of mean | 99·5 | 98·2 | 100·0 | 102·3 | 100 | 1·86 |
| Sugar content % | 12·3 | 12·6 | 12·5 | 12·3 | 12·4 | 0·14 |
| Percentage of mean | 99·2 | 101·6 | 100·8 | 99·2 | 100 | 1·17 |
| *Total sugar (cwt./acre) | 29·34 | 30·94 | 33·03 | 33·62 | 31·74 | 0·542 |
| Percentage of mean | 92·4 | 97·5 | 104·1 | 106·0 | 100 | 1·7 |
| Tops (tons/acre) | 27·51 | 27·01 | 27·81 | 28·97 | 27·82 | 0·673 |
| Percentage of mean | 98·87 | 97·09 | 99·93 | 104·11 | 100 | 2·42 |

* Treatment differences significant at $P = 0{\cdot}1\%$.

TABLE 3. 1939 *trial: eelworm infestation before treatment and after lifting (cysts, viable cysts and eggs and larvae per g.)*

| | | Control | Carbonate of lime | Calcium chloroacetate at 3 cwt./acre | Calcium chloroacetate at 6 cwt./acre | Mean | S.E. |
|---|---|---|---|---|---|---|---|
| Cysts | Before | 2·92 | 2·77 | 2·57 | 2·76 | 2·75 | 0·217 |
| | After | 3·18 | 2·88 | 2·82 | 3·16 | 3·01 | 0·301 |
| Viable cysts | Before | 0·62 | 0·59 | 0·62 | 0·53 | 0·59 | 0·065 |
| | After | 0·48 | 0·47 | 0·49 | 0·51 | 0·49 | 0·066 |
| Eggs and larvae | Before | 76 | 66 | 65 | 72 | 69 | 5·9 |
| | After | 49 | 51 | 65 | 54 | 55 | 5·1 |

*Plant population.* The treatments had a significant effect upon the number of beets per acre as deduced from the factory returns. However, this quantity is really a measure of the number of roots acceptable at the factory and is not a reliable guide to the plant population in the usual sense. $X$ and $2X$ gave significantly greater numbers than $O$ and $L$, but the $2X$ effect was not significantly greater than that of

$X$. The treatment effects remained significant even after correcting for initial variability in the eelworm infestation.

*Yield of washed beet.* The treatments had a significant effect, the $X$ and $2X$ effects being greater than that of $L$. The treatment differences were significant even after correcting for variability in the initial eelworm infestation.

The unequal plant populations might be expected to affect the validity of the results. Although no accurate estimate of plant population was made on the field, there were certainly no obvious differences between the plots receiving different treatments. It is probable that the differences recorded at the factory were due to the rejection of very small beets during the washing process. In any case, an analysis of co-variance indicated that there was no significant relation between yield and plant population. It can be concluded, therefore, that the yields were a valid measure of the treatment effects, despite the apparent discrepancy in plant populations.

*Mean weight of a single beet.* This quantity is derived from the yield of washed beet and the number of beet acceptable at the factory. The treatment differences were insignificant.

*Sugar content.* The treatment effects, as reflected in the sugar percentages, were insignificant. On the other hand, the treatment effects on total sugar were highly significant, due mainly to the effect on the yield of washed beet. The $X$ and $2X$ effects were significantly greater than that of $L$. The $2X$ effect was not significantly greater than that of $X$. The regression of total sugar on initial eelworm infestation (eggs and larvae) was significant at $P=0{\cdot}05$; nevertheless, the residual treatment effects were still significant. A separate analysis indicated that the treatment effects on total sugar were not related to the incidence of eggs and larvae after treatment.

*Eelworm infestation.* The treatment effects, as measured by cysts, by viable cysts or by eggs and larvae, were insignificant. The regression of cyst content after lifting on cyst content before treatment was significant ($P=0{\cdot}01$) but the treatment effects remained insignificant after correcting for the variability in the initial infestation. The corresponding regressions for viable cysts and for eggs and larvae were insignificant and the residual treatment effects were still insignificant.

The cyst content after lifting was significantly different from the cyst content before treatment ($P=0{\cdot}05$). The same was true for the viable cyst content ($P=0{\cdot}05$) and the egg-larva content ($P=0{\cdot}01$). It follows therefore that, whereas the cyst content increased between the two sampling dates, the viable cyst content and the egg-larva content actually decreased during the same period.

## 5. Discussion

It was evident from the results of both trials that the use of calcium chloroacetate had markedly beneficial effects on the sugar beet. From the data of the second trial it was concluded that the yields both of washed beet and total sugar were significantly increased, the responses being of the order of $1\frac{1}{2}$ tons and 4 cwt./acre, respectively. The increase in total sugar was mainly due to the effect on yield of beet rather than

to the sugar content (%). There was also a suggestion that the yield of tops was increased in both trials but this could not be confirmed. In the 1938 trial the colour of the tops was much improved and symptoms of manganese deficiency greatly reduced by the use of chloroacetate. The effects of a dressing of 6 cwt./acre were, on the whole, not appreciably greater than those of 3 cwt./acre. O'Brien *et al.* (1939) and Edwards (1939) reported similar beneficial effects on the growth of haulm and yield of tubers in potatoes.

The fact that the chloroacetate had no effect upon the eelworm infestation as measured by cysts, viable cysts or eggs and larvae brings the results into line with those of Edwards, who failed to get any reduction in the cyst content in treated potato plots. O'Brien *et al.* did not record any data for the cyst content of the soil but reported reduced larval infestation of roots in the treated plots.

The results indicated, therefore, that the beneficial effects on the sugar beet were not due to a reduction of the eelworm infestation in the treated plots. This conclusion does not rule out the possibility suggested by O'Brien *et al.* that the beneficial effect on the crop was mainly due to delayed emergence of the larvae from the cysts, the delay facilitating the establishment of the plants. On the other hand, it is possible that the improvement in the crop was a direct response to the chloroacetate, and it is clear that the effect is greater than can be attributed to the calcium content of the chloroacetate.

The eelworm data show one interesting feature which is quite independent of the treatments. The infestation, as measured by eggs and larvae (clearly the best available measure), suffered a significant decrease during the interval between samplings. The actual decrease was of the order of fourteen eggs and larvae per g., or about 20%. During the same period the number of viable cysts decreased by about 9%. Otherwise expressed, there was an increase of about 16% in the number of non-viable cysts. This can only mean that, after an initial period during which fresh cysts were produced, some factor or factors affected the viability of the cysts and resulted in a state of affairs in which a greater number of cysts included fewer viable cysts and fewer eggs and larvae. The actual factors involved are not clear but the following suggestion can be made. The crop was lifted late in September at about the time when beet lifting normally starts (the bulk of the commercial crop is, of course, lifted at a much later date). It is possible that the lifting happened to occur at a time when the cysts formed during the summer had been largely emptied of larvae, but before the resulting invasion of the roots had produced sufficient mature cysts to compensate for the earlier decrease in egg-larva content. Recent work (unpublished) by Mr F. G. W. Jones lends some support to this possibility and field observations also suggest that a flush of white cysts (i.e. fresh cysts) in the summer is followed after an interval by another flush during the autumn. However, whatever the explanation, it is clear that despite (or because of) the sugar beet there was a significant decrease in the infestation and this decrease was not due to the application of calcium chloroacetate or carbonate of lime.

The investigation formed part of a programme of work carried out on behalf of the Sugar Beet Research and Education Committee of the Ministry of Agriculture and Fisheries. I wish to acknowledge my indebtedness to the Committee and also to Messrs F. R. Petherbridge, F. G. W. Jones, K. P. Humphries, R. Chaudhuri, S. A. Khan, Dr J. Wishart, the British Sugar Corporation and Imperial Chemical Industries Ltd.

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(*Received* 26 *November* 1946)

# SOME OBSERVATIONS ON DAMAGE TO POTATOES BY SLUGS

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The garden slug, *Arion hortensis* Fér., and the keeled slugs, *Milax* spp., are the slugs mainly responsible for primary damage to potato tubers in the south-west of England. A variety trial clearly demonstrated the existence of significant differences in susceptibility to slug attack between the three varieties tested. Arran Banner was the most damaged variety, Arran Peak the least, while Majestic occupied an intermediate position. Varietal differences in the amount and time of damage to tubers were associated with differences in the dates of maturity, and little damage occurred prior to the maturing of the tuber. The results confirm the need for prompt lifting of the potato crop on maturity, in order to avoid damage by slugs.

## Introduction

The species of slugs damaging potato tubers fall into two classes: first, those which initiate damage, and secondly, those which cause merely secondary damage after the tuber has already been penetrated by another slug or insect pest. Growers and advisory officers seem generally agreed that in England the most important slug pests of the primary class are the keeled slugs of the genus *Milax* and the garden slug *Arion hortensis* Fér. These species are particularly abundant in small holdings and other horticultural land. In south-east Scotland, however, it would appear that *A. circumscriptus* Johnst. is responsible for much of the primary damage to tubers (Esslemont, 1938). In England this species is more common on grassland and waste places than on well-cultivated land.

## Preliminary observations

To determine which species were at least potentially capable of primary damage to potato tubers two preliminary experiments were made in 1943 and 1944. Nine species of common slugs were tested. Twenty-four well-grown individuals of each species were placed separately in 10 in. flower pots with damp soil (free from slugs) containing one Arran Banner tuber per pot. The drainage holes and tops of the pots were covered to prevent the escape or ingress of slugs. No food other than the potato was provided, and at the end of 1 month the pots were emptied out and the tubers examined for slug damage. The results are shown in Table 1. It is interesting to note that all the *A. subfuscus* Drap. and *A. ater* L. were dead at the end of the month and that cannibalism had occurred in all three species of *Milax*.

A subsequent experiment showed that *Agriolimax reticulatus* Müll. and *Arion circumscriptus* will eat their way into any cut or damaged portion of a tuber, but rarely bore through a perfectly healthy skin.

The results of these tests taken in conjunction with the general experience of other workers make it clear that *A. hortensis* and the species of *Milax*, especially *M. gracilis* Leydig and *M. sowerbyi* Fér., are the slugs mainly responsible for damage to potato tubers. Secondary damage, following damage by one or all these species, by *Arion reticulatus*, millepedes and woodlice is common and widespread.

TABLE 1. *Pot experiments indicating damage to potato tubers by some common species of slugs*

| Species | 1943 | 1944 |
|---|---|---|
| *Agriolimax reticulatus* | None | None |
| *Arion hortensis* | Severe | Severe |
| *A. intermedius* | None | Very slight |
| *A. circumscriptus* | Slight | Very slight |
| *A. subfuscus* | None | None |
| *A. ater* | None | None |
| *Milax gracilis* | Very severe | Very severe |
| *M. sowerbyi* | Very severe | Very severe |
| *M. gagates* | Severe | Severe |

Among the numerous substances which have been claimed from time to time to destroy or deter slugs and snails, there can be no doubt that metaldehyde is the most efficient. Its superiority over Paris Green in this respect has already been demonstrated (Thomas, 1943), and a considerable amount of information has been obtained concerning the correct use and effect of this substance (Barnes & Weil, 1942; Jary & Austin, 1937; Esslemont, 1938).

However, certain experiments with metaldehyde against the keeled slugs of the genus *Milax* have led the author to suspect that metaldehyde in its present known form of application would kill only a relatively small number of these slugs present under a growing crop of potatoes. It is hoped that the full details of these experiments will be published later.

## VARIETAL TRIAL

An interesting case of the apparent immunity of the potato variety Arran Peak was reported in November 1943 by Mr G. Fox Wilson. Plants of this variety growing alongside those of Dunbar Standard, Red King and Golden Wonder escaped the damage by *Milax* spp. from which other varieties suffered severely.

A trial was set up, on the Horticultural Department land at Seale-Hayne College, to investigate this apparent immunity more exactly.

### *Lay-out of experiment*

Three varieties were chosen, Arran Banner, Majestic and Arran Peak, giving a wide range of dates of maturity of the tubers. A plot known to be infested both with *Arion hortensis* and *Milex gracilis* was selected for the experiment and divided into three equal parts, each 18 ft. wide and 21 ft. long. On 27 March 1944, setts of the three varieties were planted in nine rows each of fourteen setts. Arran Peak

was planted in the plot next to the hedge, Majestic in the middle and Arran Banner in the top plot adjoining a pathway.

It was considered essential in a varietal trial of this kind to have the varieties in blocks containing only one variety, and that these blocks should be large enough to minimize the effect of the counter-attraction of one variety against any other variety. This type of experimental lay-out should be of value in many trials of resistance or susceptibility to insect attack where the pest is able to exert a free choice of either feeding or oviposition. For instance, where the pest concerned is freely mobile, such as the potato-leaf hopper or potato aphides, it becomes almost impossible to assess the differential injury in the field (Allen & Rieman, 1939; Burnham & Macleod, 1942). To obtain a valid estimate of the performance of any one variety it becomes necessary to maintain a known artificial infection on pure stands of the variety under controlled conditions, or to know the potential population of the pest on a field scale, again on pure stands of the variety. In this particular trial the relative immobility of slugs, the size of the experimental plot, and the fact that the relative slug populations could be estimated during the experiment, was assumed to be sufficient to enable valid comparisons between the varieties to be made.

TABLE 2. *Estimates of the slug population on three experimental plots*

| | Total no. of slugs caught by meta-bran bait 14 April | | No. of slugs seen on lifting 2 November | | No. of slugs found in soil samples 6 November | | No. of slugs caught 16 November | |
|---|---|---|---|---|---|---|---|---|
| Species | *A* | *M* | *A* | *M* | *A* | *M* | *A* | *M* |
| Arran Banner | 10 | 16 | 55 | 10 | 1 | 4 | 23 | 6 |
| Majestic | 16 | 19 | 41 | 11 | 2 | 7 | 29 | 14 |
| Arran Peak | 16 | 26 | 73 | 14 | 4 | 4 | 36 | 12 |

*A* = *Arion hortensis*. *M* = *Milax gracilis*.

Owing to unfavourable weather conditions it was not possible to obtain an estimate of the relative abundance of slugs on the three plots until 14 April. On that date six slug baits, consisting of metaldehyde and bran in the proportions of 1 to 10 by volume, were placed on each of the three plots. The examination of the baits on the following morning showed that there was a marked gradient of slug population, particularly of *M. gracilis*. The Arran Peak plot was the most heavily infested and the Arran Banner plot the lightest, while the Majestic plot was intermediate. The figures are shown in Table 2, together with subsequent counts. After the traps had been examined they were removed to prevent any unwanted reduction in the slug population on the plots.

Of the plants in each plot, only an inside block of $5 \times 10$ plants was considered in the estimation of relative slug damage. This gave a protective ring, two plants wide, round each of the plots, the purpose of which was to intercept migrating

slugs which might be attracted to one or all of the plots from neighbouring crops of other vegetables.

### *Estimation of slug damage*

In each varietal block two plants in each of the five inside rows were selected at random, lifted, and the tubers of ware and seed size examined for signs of slug damage. Only penetrating holes were regarded as damage, and any tuber with one or more holes was counted as a damaged tuber. When the number of clean and damaged tubers had been recorded for each plant separately, the tubers were all replaced in the holes from which they had been taken and covered with soil again. In this way the number of tubers in each plot remained constant, and there was less chance of an apparent increase in percentage damage at later counts due to the reduction in the amount of available food per slug.

Ten plants of each variety were lifted on each occasion. The sampling began on 7 September and continued at fortnightly intervals to 2 November, when all the plants in the experimental blocks were lifted and removed to the laboratory, washed, and the damaged and undamaged tubers weighed to the nearest pound.

TABLE 3. *Percentage potato tubers damaged by slugs on five dates of lifting*

| Variety ... | Arran Banner | | Majestic | | Arran Peak | |
|---|---|---|---|---|---|---|
| | % damage | No. of tubers lifted | % damage | No. of tubers lifted | % damage | No. of tubers lifted |
| 7 September | 29* | 78 | 7 | 81 | 5 | 80 |
| 21 September | 48 | 88 | 14 | 88 | 7 | 74 |
| 5 October | 49 | 89 | 20* | 81 | 8 | 86 |
| 19 October | 47 | 95 | 24 | 74 | 9* | 97 |
| 2 November | 49 | 84 | 25 | 78 | 15 | 82 |
| 2 November (all plants) | 50 | 405 | 26 | 386 | 18 | 420 |

* Approximate times of ripening of the three varieties.

### *Estimation of slug population*

As the potatoes were lifted for the last time the number of slugs seen in or around the tubers was counted as a rough check on the distribution of slug population on the plots. The slug population was also checked in two other ways on the plots after the final lifting; first by soil sampling with a wireworm sampling tool taking a 4 in. diameter core of soil (these cores were then taken to the laboratory and washed through fine-mesh sieves and the slugs in them counted and identified), and secondly by metaldehyde baits, using ten baits per plot. The results are shown in Table 2, where the comparison of the populations in April and November is made.

## Discussion of results

The results summarized in Tables 2 and 3 show clearly that at the time of lifting, Arran Banner was more severely damaged than was Arran Peak. Moreover, the estimates indicate that the slug population was highest on the Arran Peak plot and lowest on the Arran Banner plot both at the beginning and end of the experiment, although it seems probable from the figures that some redistribution of slugs over the plots had occurred, especially with *Arion hortensis*.

The examination of the trap rows round the borders of the experimental plots gave the following figures: Arran Banner 57·6%, Majestic 35·0% and Arran Peak 24·8% damaged. It would appear that these trap rows effectively intercepted the extraneous slug population which was attracted to the potatoes from neighbouring plots.

Examination of the progress of the damage to the tubers on the three varieties through the season shows that on 7 September Arran Banner was already severely attacked, although Majestic and Arran Peak were nearly clean; by 21 September, Arran Banner reached 51% damage, and thereafter the percentage damage remained constant at that level. At this time Majestic was showing signs of increasing damage while Arran Peak was not significantly more damaged than on 7 September. The damage to Majestic increased in a regular manner until about 19 October, when the rate of increase slowed down and the curve of percentage damage against date showed a tendency to flatten out in a similar way to that of Arran Banner. Arran Peak showed only a slight increase in damage up to 19 October, but on 2 November the damage had increased to 15%. As the experiment was discontinued on this date it is not possible to say how far the damage to Arran Peak would have increased or whether it would eventually reach a maximum and remain constant at that figure. It is hoped, however, that when this experiment is repeated it will be possible to 'stagger' the dates of lifting for the three varieties so as to cover the whole period for each from the onset of slug attack to its final phase.

The most significant feature of the time of the attack of the tubers is that it corresponds roughly with the time of ripening of the tubers. Thus Arran Banner appeared to be mostly ripe by 7 September, Majestic by 5 October and Arran Peak by 19 October. This association of attack with maturity of tuber agrees with field observations and the general impression among growers that main crop potatoes have to be lifted before the autumn rains set in and slugs become active. As this date of lifting would correspond roughly with the date of maturity it seems likely that the reason they escape damage is not that the slugs are inactive in the late summer, but that the potatoes are not in a condition attractive to the slugs. Similar observations have been made (Anon., 1944) in the relation of wireworm damage to tubers and their date of maturity.

Investigations are proceeding to attempt to determine the fundamental causes for the unattractiveness or unpalatability of unripe potatoes to slugs, and also to

discover what practical steps could be taken to defer the date of maturity, thus increasing the period of growth without exposing the tubers to the attacks of slugs and wireworms.

Grateful acknowledgement is made to Mr L. N. Staniland, Advisory Entomologist, South-Western Province, for his ever-ready assistance and advice, and to Dr H. F. Barnes and Mr J. W. Weil, of Rothamsted Experimental Station, who took a keen interest in the initiation and progress of this work, and finally to Mr O. D. Kimble for his kindness in allowing the use of the Horticultural Department land at Seale-Hayne College for the experimental plots.

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(*Received* 6 *November* 1946)

# THE COMPETITION BETWEEN BARLEY AND CERTAIN WEEDS UNDER CONTROLLED CONDITIONS

## II. COMPETITION WITH *HOLCUS MOLLIS*

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(With Plate 5)

The nature of the competition between barley and the twitch grass, *Holcus mollis*, has been studied. *H. mollis* has overrun much of the light, semi-acid arable land near Woburn on Lower Greensand soils; about 2½ tons of dry rhizomes and roots per acre were dug up on land where it had grown for 10 months. All this rhizome growth occurred within the top 8 in. of the soil. *H. mollis* commonly increases until the roots occupy from 0·11 to 0·14 l. root space per 1 g. dried roots and rhizomes. It is slightly superior to barley in ability to utilize available nitrogen.

When barley and this *Holcus* are grown together with abundance of food and water for both plants, the effect of the weed on the yield of barley depends on whether the barley or the weed first becomes established and on the rate of sowing of the barley. If barley growth begins before that of *Holcus*, it may smother the weed completely and sustain no loss of yield provided the barley is planted thickly enough. If the two competing plants start at the same time, thickness of planting is still able to limit the extent to which the weed can reduce the yield of barley, though even with the thickest planting the *Holcus* can reduce barley yield by one-third. Where *Holcus* is already established in the ground from the previous year and the crop is thinly planted, the weed is able to eliminate the barley completely, but where barley is sown thickly it is still able to give a 50% yield.

With a sparse crop of barley, an increase in the number of weed units makes no difference to the growth of the barley provided the crop becomes well established. Where the crop and the weed start together and so compete on equal terms, any increase of *Holcus* growth is obtained at the cost of an almost equal falling off in the yield of barley, showing that the respective plant growths are almost complementary. Once the weed has fully occupied the soil, it is able to suppress the growth of the barley almost entirely. The barley and the *Holcus* reduce the growth of each other even when the root space and supply of plant food are ample for both.

### Introduction

In a paper published two years ago (Mann & Barnes, 1945), we gave an account of experiments on the competition between barley and two of the weeds common on light semi-acid land in Bedfordshire. These weeds, *Spergula arvensis* and *Matricaria inodora*, are perhaps the most important and certainly the most abundant among the annual weeds appearing in barley under the conditions in question. But in agricultural importance they are far less serious than the perennial weeds and particularly than the grasses with underground rhizomes, commonly called twitch or couch grass. In the soil on which we have worked, the twitch which is usually considered as typical of these grasses, *Agropyrum repens*, hardly occurs, but it is

replaced by two others which are equally difficult to deal with and which are very common indeed. These are *Holcus mollis* and *Agrostis stolonifera*, both of which are liable to infest land otherwise suitable for barley and which seem to reduce very materially the crop of grain which is produced. It is difficult to say which of these two grasses is the more frequent or the more serious. Both are common and both are bad. The object of the present work has been to find out the actual effect of such competition with each of them, and we have so far been able to study that with *Holcus mollis*, using the methods already employed in connexion with the annual weeds mentioned above.

## Characteristics of *Holcus mollis*

On the area of lower greensand soils on which Woburn lies, *Holcus mollis* has long been recognized as a serious pest. In 1808, Batchelor stated that so far as the light soils of Bedfordshire are concerned, 'the creeping soft grass (*H. mollis*) is by far the most common'. Sinclair (1824), dealing with the grasses in the neighbourhood of Woburn, speaks of the grass as follows: 'The roots, when once in possession of the soil, can hardly be expelled without great labour and expense. It is the true couch of light sandy soils. I have found roots 5 ft. in length, the growth of a few months.' He further states that the roots when ground up have the flavour of new made meal and that pigs are very fond of the creeping roots and 'dig them up with eagerness'. He considered that the only way to get rid of the weed was to apply yearly sufficient dressings of clay, 'perhaps fifty loads per acre', till the texture of the soil was changed. In 1861, Sowerby & Johnson considered it as one of the most troublesome weeds on sandy soils and as very difficult to eradicate 'in consequence of its rapid growth and unduly creeping habit'.

The roots of this grass were examined by Grainger (1935) as it grew on acid land near Huddersfield. He found that the rhizomes were all within 6 in. of the surface. One square foot of ground gave 110 ft. length of rhizomes. As 19½ in. length of rhizome bore 10 ft. length of fibrous roots, there was no less than 770 ft. length of total roots within a square foot of ground. Most of the fine roots ramified in the surface layers of the soil along with the rhizomes.

Our own study of the roots in the field was done on a plot infested with the weed where wheat had failed owing to acidity of the soil. Excavation was done in June and again in September when *Holcus*, which had grown from fresh fragments of the rhizomes the same year, was, in June, about 1 ft. high with the flowers just beginning to show, and, in September, was carrying fully ripened seed. Below 8 in. deep, the compact soil was devoid of rhizomes and only carried a small amount of fibrous roots. Above this, the quantity of *Holcus* roots was determined at two depths, i.e. from the surface to 4 in. deep, and from 4 to 8 in. deep. The amount of roots per acre at each of the two depths at the two dates specified is shown in Table 1.

TABLE 1

| | Roots in June | | | Roots in September | | |
|---|---|---|---|---|---|---|
| | 4 in. depth | 4–8 in. depth | Total | 4 in. depth | 4–8 in. depth | Total |
| Wt. of roots (lb.) per acre: | | | | | | |
| (1) As dug up | 3576 | 700 | 4276 | 14,130 | 3020 | 17,150 |
| (2) Dry matter | 880 | 163 | 1043 | 4,580 | 992 | 5,572 |
| Nitrogen in dry roots (%) | 0·49 | 0·72 | | | | |
| Ash in dry roots (%) | 12·99 | 9·55 | | | | |

The rapidity of the growth of the underground portion of *H. mollis* is very remarkable. The land had been cleared of growing roots in the previous November, so that the above was the result, in September, of only 10 months' growth, but gave over $7\frac{1}{2}$ tons of material as dug up or $2\frac{1}{2}$ tons of dry matter within the top 8 in. of the soil.

Generally speaking, this grass has been specially prominent at Woburn on the more acid plots where the *p*H value is below 4·8, and even where it is as low as 4·2. Here it grew with great luxuriance, but it also appeared very strongly in the ordinary soil of the farm where the *p*H value is about 6·0.

## METHOD OF STUDY

The importance of this twitch under the conditions indicated made it worth while to study the effect of one *Holcus* plant on another where space was known and limited, and also the way in which it competed with barley under various relative thicknesses of planting both of the barley and of the weed. Both barley and weed were grown in earthenware pots, 28 cm. in diameter and 25·5 cm. deep, furnished with an upturned outlet near the bottom, which enabled watering to be done without danger of loss and yet secured good aeration of the soil. The bottom of the pot was covered with coarse gravel to above the outlet. Each pot was then filled with 16·3 kg. of soil taken from one of the fields at Woburn. This gave about 20 cm. depth of actual soil without counting the gravel at the bottom of the pot. Each pot contained, therefore, 12·3 l. occupied by soil, and if the volume of the gravel be included in that available to the plant roots, the whole volume would be 14·2 l. per pot. This space remained constant during the experiments. Sufficient potassium phosphate was added to supply amply all the phosphates and potash the plants would require, and, in addition, enough nitrogen in the form of ammonium sulphate to make it certain that neither the barley nor the weed when grown alone lacked nitrogen during the period of growth. The pots were kept well supplied with water during the growth of the crop and the weed, but at the same time the soil was always well aerated. The only variables in the experiment were, therefore, the number of barley or of *Holcus* plants for a definite volume of soil.

Before actually conducting the competition experiments, it was necessary to determine the amount of nitrogenous manure that should be added per pot so as

to ensure that there would be no nitrogen stress. In previous work (Mann & Barnes, 1945) we have shown that, with barley alone, this amounted to 1·25 g. nitrogen per pot at least, and that even with this amount it was doubtful whether a full development of the barley had been reached. We have now been able to carry these tests further, even up to an addition of 1·75 g. nitrogen per pot. It was quickly evident, however, that at 1·25 g. nitrogen per pot (i.e. at 0·09 g. nitrogen per litre of root space) we had reached a full yield of both barley grain and straw and that a still higher quantity did not result in an increase of yield but only in a larger percentage of nitrogen in the produce. Table 2, with various thicknesses of barley, shows this. The addition of the extra amount of

TABLE 2. *Results of increasing nitrogen supply on yield of barley*

| No. of barley plants per pot | Root space per plant (l.) | Yield of grain per pot (g.) | | Yield of total produce (above ground) per pot (g.) | | Nitrogen in dry matter of produce (g.) | |
|---|---|---|---|---|---|---|---|
| | | 1·25 g. nitrogen | 1·75 g. nitrogen | 1·25 g. nitrogen | 1·75 g. nitrogen | 1·25 g. nitrogen | 1·75 g. nitrogen |
| 1 | 14·2 | 53·6 | 54·3 | 144·5 | 138·4 | 0·82 | 0·94 |
| 2 | 7·1 | 69·8 | 66·1 | 156·5 | 151·1 | 0·78 | 0·90 |
| 4 | 3·5 | 65·7 | 70·4 | 152·3 | 151·1 | 0·73 | 0·87 |
| 6 | 2·4 | 73·6 | 68·7 | 163·7 | 151·4 | 0·72 | 0·87 |
| 8 | 1·8 | 77·3 | 66·3 | 166·2 | 141·0 | 0·74 | 0·89 |
| | Mean | 68·0 | 65·2 | 156·6 | 146·6 | 0·76 | 0·89 |

TABLE 3. *Effect of thickness of planting on yield of* Holcus

| No. of *Holcus* plants per pot | Root space per plant (l.) | Maximum no. of *Holcus* shoots per plant | Wt. of air-dry *Holcus* (above ground part) per pot (g.) | | Wt. of air-dry *Holcus* roots per pot (g.) | | Mean of 2 years | |
|---|---|---|---|---|---|---|---|---|
| | | | 1944 | 1945 | 1944 | 1945 | N % | Root space per 1 g. root (l.) |
| 1 | 14·2 | 6·5 | 36·0 | 93·6 | 31·5 | 73·0 | 1·36 | 0·27 |
| 2 | 7·1 | 3·5 | 31·8 | 107·7 | 42·8 | 83·3 | 1·28 | 0·23 |
| 4 | 3·5 | 5·5 | 51·9 | 127·1 | 78·4 | 88·1 | 0·97 | 0·17 |
| 6 | 2·4 | 4·7 | 58·4 | 113·6 | 73·9 | 87·3 | 0·96 | 0·18 |
| 8 | 1·8 | 7·4 | 74·8 | 101·6 | 101·1 | 111·6 | 0·93 | 0·13 |

nitrogen has not increased the yield of either the grain or the total produce, at any thickness of planting, but it has given a crop containing 17% more nitrogen. It would seem fair to state that at the standard of application suggested and used (1·25 g. nitrogen per pot) there is no nitrogen stress, and that under these conditions the only competition between the barley plants is for root space. Further, an increase in the number of barley plants per pot beyond four (or an allowance of 3·5 l. of root space per plant) will not increase the crop provided there is no stress for nitrogen at any stage in the life of the barley plant.

Using the same application of nitrogen to *Holcus* plants grown alone, the effect of thickness of planting on the yield of produce in 1944 and 1945, using *Holcus* planted the same spring, is given in Table 3. These figures show that with plants of

*H. mollis* in the first year, the stress for root space (plenty of nitrogen being present) does not begin till the soil volume per plant is less than 3·5 l. *H. mollis* is, however, a perennial, and, as a weed, is usually far more serious in the second year than in the first after the land becomes infested. Therefore the plants in the above experiment in 1944 were allowed to stay in the pots for the winter and then to grow with a further addition of 1·75 g. of nitrogen as ammonium sulphate per pot. Under these circumstances the *Holcus* grew greatly, filled the pots with rhizomes and roots and gave the yields shown in Table 4.

TABLE 4. *Effect on yield of leaving* Holcus mollis *for a second year*

| No. of *Holcus* plants per pot | Root space per plant (l.) | Wt. of air-dry *Holcus* (above ground part) per pot (g.) | Percentage increase from 1st to 2nd year | Wt. of air-dry *Holcus* roots per pot (g.) | Percentage increase from 1st to 2nd year |
|---|---|---|---|---|---|
| 1 | 14·2 | 144·0 | 300 | 101·0 | 221 |
| 2 | 7·1 | 138·2 | 335 | 99·0 | 131 |
| 4 | 3·5 | 147·2 | 184 | 150·3 | 92 |
| 6 | 2·4 | 163·6 | 180 | 133·0 | 80 |
| 8 | 1·8 | 148·1 | 98 | 100·2 | −1 |
| | Mean | 148·2 | | 116·7 | |

Three things seem clear from the figures given in Table 4:

(1) The roots of *H. mollis*, whether derived from one plant or from a larger number, tend to increase in the ground till they occupy from 0·11 to 0·14 l. per gram of air-dry roots. This seems the limit, and in no case was this amount of root increased even though there was plenty of nitrogen and water present.

(2) Whatever the number of plants originally present, the amount of growth above ground in the second year did not vary directly with the number of plants when the root space was controlled.

(3) In the first year, excess of nitrogen in the soil presents itself in a higher nitrogen content in the produce. When, however, the maximum growth of both roots and tops is attained, a constant nitrogen content is found in the produce.

There is one point with which we have not yet dealt in regard to the relative activity of barley and *H. mollis* when grown alone, in each case. By some workers it has been suggested that competition between crop plants and weeds is usually a competition for nitrogen, and this renders it of interest to see the use that this crop and this weed, grown alone at various densities of plant, can make of a sufficient supply of this when applied in the form of ammonium sulphate. Table 5 shows that the capacity of barley and *H. mollis* to take advantage of the available nitrogen does not seem to differ very much. But while with barley by far the greater part of the nitrogen appears in the above-ground portion of the plants, with *Holcus* almost half is retained in the roots and underground rhizomes of the plant.

TABLE 5. *Nitrogen recovered (mg.) in barley or* Holcus mollis *grown alone*

| No. of barley or *Holcus* plants per pot | Barley grown alone | | | *Holcus mollis* grown alone | | |
|---|---|---|---|---|---|---|
| | Above-ground portion | Roots alone | Total | Above-ground portion | Roots alone | Total |
| | A. 1944. Nitrogen added 1250 mg. per pot | | | | | |
| 1 | 997 | 56 | 1053 | 548 | 413 | 961 |
| 2 | 987 | 58 | 1045 | 449 | 485 | 934 |
| 4 | 1198 | 104 | 1302 | 542 | 713 | 1255 |
| 6 | 1227 | 115 | 1342 | 545 | 607 | 1152 |
| 8 | 1244 | 123 | 1367 | 690 | 944 | 1634 |
| Mean | 1131 | 91 | 1222 | 555 | 632 | 1187 |
| | B. 1945. Nitrogen added 1250 mg. per pot | | | | | |
| 1 | 1042 | 109 | 1151 | 767 | 488 | 1255 |
| 2 | 1074 | 124 | 1198 | 852 | 542 | 1394 |
| 4 | 975 | 125 | 1100 | 798 | 622 | 1420 |
| 6 | 1037 | 144 | 1181 | 812 | 560 | 1372 |
| 8 | 1078 | 172 | 1250 | 688 | 615 | 1303 |
| Mean | 1041 | 135 | 1176 | 783 | 565 | 1349 |
| | C. 1945 (soil used in previous year). Nitrogen added 1750 mg. per pot | | | | | |
| 1 | 1135 | 123 | 1258 | 1122 | 676 | 1798 |
| 2 | 1189 | 112 | 1301 | 912 | 840 | 1752 |
| 4 | 1143 | 148 | 1291 | 987 | 1292 | 2279 |
| 6 | 1157 | 189 | 1346 | 1053 | 951 | 2004 |
| 8 | 1107 | 198 | 1305 | 967 | 870 | 1837 |
| Mean | 1146 | 154 | 1300 | 1008 | 926 | 1934 |

Taking the mean of all thicknesses of plants in the soil, we find that with barley, grown with an addition of 1·25 mg. of nitrogen as fertilizer, the total nitrogen recovered is remarkably even. Even with the higher nitrogen dressing under C it is little increased, while of the total only 11·8% is contained in the roots. With *Holcus*, the total amount of nitrogen recovered is little different from that with the barley where, as in A and B, the *Holcus* was planted in the same year, but that contained in the roots is 47% of the total. Where, as in C, the *Holcus* has remained in the soil from the previous year and really consists of 2 years' growth, the recovery of nitrogen is greater, but the proportion contained in the roots is similar (48%).

## COMPETITION BETWEEN BARLEY AND *HOLCUS MOLLIS*

So far we have dealt with the amount of barley or *Holcus* growth that can be made in a limited space without the competition of any other plant. We have now to consider the way in which the advent of this weed on a soil where barley is being grown will lower the yield of the barley or, on the other hand, how far a crop of barley will be able to smother the weed under study. Two cases at once arise. First, what will be the effect of an increase in the thickness of the barley sowing when the weed infestation remains constant; secondly, what will be the effect of

an increase in the amount of weed infestation when the stand of barley remains the same? We will consider the two cases separately.

Before doing so, however, we may say that in studying the roots of the two plants when grown together, we have, whatever be the proportion of the two, been unable to find any special relationship between them. The roots form a combined mass so closely interwoven that it is impossible to separate them (Pl. 5, fig. 2). There is no sign of any repulsion between the roots of the barley and those of the weed, nor is there any sign of the opposite. They compete for the occupation of a limited space when the amount of available nitrogen is sufficient for both, and the one which will succeed in this competition will be simply that which gets the best start in the season in question.

### I. *Weed infestation constant: barley thickness variable*

In all cases under this head in our experiments, there were six plants of *Holcus* per pot, while the number of barley plants varied from one to eight. Thus there was a variation between a sparse plant of barley and a very thick seeding. The *Holcus* was transplanted from the field either a few days after the barley was sown or at the same time as the barley seed was put in. In all cases one effective joint of rhizome was planted to make one unit of the weed. Thus we have a comparison of the effect when the barley gets a good start on the weed, and when the two start more or less level. A third case occurs when the *Holcus* is already well established in the soil: this is arranged for by leaving the growing *Holcus* in the soil for one year, thus giving it a good chance to get its root system well established before the planting of the barley. The results on the growth of both the barley and the *Holcus* with varying thicknesses of barley, in each of the three cases, is shown in Table 6.

When *Holcus* starts growth 8 or 10 days after the sowing of the barley as in § A of Table 6, the effect of the weed is very slight on the yield of that year. Judged by the maximum number of shoots on the barley plants in the spring, there is only a very small reduction, the tillers per plant giving a mean of 26 without *Holcus* and 24 with the weed. When the number of barley plants is small relative to the number of *Holcus*, the reduction of shoots is considerable; when the numbers of the two plants are anything like equal, there is no effect at all. Almost the same may be said of the final yield. When the number of barley and *Holcus* plants is equal or when the number of barley plants is in excess, the yield of grain is not at all affected, though the straw is slightly reduced. Further, the presence of a thick barley sowing prevents the *Holcus* plants from growing, as is shown by the steady reduction in the weight of both the above-ground growth and the roots of the twitch as the number of barley plants increases. It is clear, in fact, that with *Holcus* plants which begin to grow when the barley is already well germinated, the effect of increasing the number of barley plants (the number of weed plants remaining constant) is to diminish the injurious action of the weed until with six or eight barley plants per pot there is no reduction in the yield of the barley. The whole result is in accordance

with the idea that a thick seeding of a cereal crop will tend to smother weeds, even twitch, provided the twitch is a new infection. The growth of the weed above ground is reduced by more than 75% when the number of barley plants per pot is increased from one to eight.

TABLE 6. *Yield (g.) of barley and* Holcus *with varying amounts of barley*

| No. of plants per pot | | Maximum no. of barley shoots per plant | Yield of barley per pot | | Yield of total produce per pot | | Yield of roots per pot | |
|---|---|---|---|---|---|---|---|---|
| Barley | *Holcus* | | Grain | Total produce | *Holcus* (above ground) | Barley and *Holcus* together | Barley | *Holcus* |
| | | | A. Barley sown 9 days before *Holcus* | | | | | |
| 0 | 6 | — | — | — | 58·4 | 58·4 | — | 73·9 |
| 1 | 6 | 36 | 24·2 | 66·4 | 31·4 | 97·8 | 2·4 | 33·5 |
| 1 | 0 | 45 | 41·7 | 112·4 | — | 112·4 | 4·0 | — |
| 2 | 6 | 31 | 40·8 | 98·1 | 24·0 | 122·1 | ? | ? |
| 2 | 0 | 35 | 41·2 | 102·9 | — | 102·9 | ? | ? |
| 4 | 6 | 23 | 49·2 | 119·4 | 16·0 | 135·4 | 5·8 | 16·0 |
| 4 | 0 | 20 | 65·7 | 155·2 | — | 155·2 | 7·5 | — |
| 6 | 6 | 17 | 62·3 | 142·5 | 9·0 | 151·5 | 7·3 | 6·5 |
| 6 | 0 | 16 | 63·8 | 152·2 | — | 152·2 | 7·8 | — |
| 8 | 6 | 14 | 66·8 | 153·8 | 7·1 | 160·9 | 9·5 | 3·9 |
| 8 | 0 | 13 | 63·1 | 153·7 | — | 153·7 | 9·5 | — |
| | | | B. Barley sown and *Holcus* planted at the same time | | | | | |
| 0 | 6 | — | — | — | 113·6 | 113·6 | — | 87·3 |
| 1 | 6 | 15 | 20·9 | 47·0 | 83·5 | 130·5 | 3·1 | 121·8 |
| 1 | 0 | 44 | 53·6 | 144·5 | — | 144·5 | 9·4 | — |
| 2 | 6 | 15 | 28·4 | 68·0 | 78·6 | 146·6 | 5·7 | 132·8 |
| 2 | 0 | 26 | 69·8 | 156·5 | — | 156·5 | 13·0 | — |
| 4 | 6 | 12 | 38·6 | 87·7 | 58·3 | 146·0 | 7·3 | 131·6 |
| 4 | 0 | 15 | 65·7 | 152·3 | — | 152·3 | 12·6 | — |
| 6 | 6 | 10 | 51·0 | 116·0 | 44·0 | 160·0 | 11·9 | 80·7 |
| 6 | 0 | 13 | 73·6 | 163·7 | — | 163·7 | 16·8 | — |
| 8 | 6 | 8 | 50·0 | 111·6 | 50·3 | 161·9 | 14·9 | 81·1 |
| 8 | 0 | 9 | 77·3 | 166·2 | — | 166·2 | 22·1 | — |
| | | | C. Barley sown on land containing 1 year's *Holcus* roots | | | | | |
| 0 | 6 | — | — | — | 163·6 | 163·6 | — | 133·0 |
| 1 | 6 | 1 | 1·3 | 3·0 | 194·2 | 197·2 | 0·2 | 136·2 |
| 1 | 0 | 43 | 54·3 | 138·4 | — | 138·4 | 9·9 | — |
| 2 | 6 | 2 | 3·1 | 6·4 | 176·9 | 183·3 | 0·5 | 119·8 |
| 2 | 0 | 21 | 66·1 | 151·1 | — | 151·1 | 11·3 | — |
| 4 | 6 | 2 | 9·8 | 19·8 | 155·1 | 174·9 | 2·0 | 101·4 |
| 4 | 0 | 12 | 70·4 | 151·1 | — | 151·1 | 15·0 | — |
| 6 | 6 | 3 | 13·6 | 29·5 | 130·1 | 159·6 | 3·7 | 80·4 |
| 6 | 0 | 9 | 68·7 | 151·4 | — | 151·4 | 19·2 | — |
| 8 | 6 | 4 | 28·6 | 59·4 | 96·1 | 155·5 | 8·0 | 86·1 |
| 8 | 0 | 7 | 66·3 | 141·0 | — | 141·0 | 18·9 | — |

These results are considerably modified when the *Holcus* and the barley get an even start together, the barley seed being sown and the plants of *Holcus* being

planted at the same time, as shown in § B of Table 6. The *Holcus* was, in fact, very much more vigorous than in the previous case, the competition between it and the barley was more marked, and the increase in the number of barley plants from one to eight did not entirely neutralize the effect of the weed. With the maximum number of barley plants per pot (eight) the effect of the six plants of *Holcus* on the early growth of the barley (as judged by the tillering) was only small, but it was able to reduce very markedly the yield of both grain and total produce. There is, however, a general reduction in the effectiveness of the competition as the number of barley plants gets larger, though even with the thickest sowing of barley the loss in yield is over 30% both in grain and in total produce.

The way in which the growth of barley may be almost completely prevented when *Holcus* has really got the chance to take previous possession of the ground is shown in § C of Table 6. Under these conditions it is able to eliminate the barley almost entirely unless the latter is sown very thick.

The actual reduction in yield of barley due to the presence of six *Holcus* plants per pot, with varying thicknesses of the crop, is shown in Table 7, under the three conditions of relative time of sowing the barley and planting the *Holcus*.

TABLE 7. *Percentage reduction in yield of barley due to six plants of* Holcus *per pot*

| No. of barley plants per pot | A (Barley sown before *Holcus*) | | B (Barley and *Holcus* sown same time) | | C (*Holcus* already established) | |
|---|---|---|---|---|---|---|
| | Grain | Total produce | Grain | Total produce | Grain | Total produce |
| 1 | 42 | 41 | 61 | 68 | 98 | 98 |
| 2 | ? | ? | 59 | 57 | 95 | 96 |
| 4 | 25 | 23 | 41 | 42 | 86 | 87 |
| 6 | 2 | 6 | 31 | 29 | 80 | 80 |
| 8 | 6* | Nil | 35 | 33 | 57 | 58 |

* Increase.

The effect of the increasing thickness of the barley plant on the growth of *Holcus*, so far as the above-ground portion of the latter is concerned, is equally striking (Table 8).

TABLE 8. *Percentage reduction in growth of* Holcus *due to increasing thickness of barley plants*

| No. of barley plants per pot | A (Barley sown before *Holcus*) Total produce | B (Barley and *Holcus* sown same time) Total produce | C (*Holcus* already established) Total produce |
|---|---|---|---|
| 1 | 46 | 26 | 19 (increase) |
| 2 | 59 (?) | 31 | 8 (increase) |
| 4 | 73 | 49 | 5 |
| 6 | 85 | 61 | 20 |
| 8 | 88 | 56 | 41 |

The way in which a thickly sown crop of barley is able to prevent the growth of *Holcus*, provided it gets a fair start, is shown by the first column of Table 8, where there is a reduction of 88% in the above-ground growth of this weed; where the two are sown or planted at the same time, this reduction is much smaller, being only 56%; where *Holcus* is already established and the barley plants are sparse they actually show an increase in the amount of weed growth, but when the barley plant is thick, it is, even in this case, able to reduce the growth of *Holcus* by 41%.

When we attempt to study the effect of the competition on the amount of root development, we are met by the difficulty that it is impossible to separate the roots of barley and those of *Holcus* when they are grown together, and so the whole weight of the combined root growth only can be taken. The amount of barley root in this combined figure can be approximately obtained by calculating the amount of root corresponding to the amount of top growth, and so the relative quantity of the two roots in the combined mass is obtained. The results of this calculation are shown in the last two columns of Table 6. It is not claimed that the figures are exact, but they will give some idea of the effect of the relative amount of the two plants on the root development of each. They show that when the barley gets a start on the weed, even a very small amount of barley relative to *Holcus* is able to reduce largely the root development of the latter, and when the sowing of the barley is thick it is able almost to eliminate it. When the two competing plants are put into the ground at the same time, the amount of root development in *Holcus* is not affected till the number of plants of barley is approaching that of the weed. When the number is actually equal or when the number of barley plants exceeds that of *Holcus*, there is a substantial reduction in the root development of the weed by about 33%. Again, when *Holcus* is already well established in the soil, as in § C, a thick sowing of barley is able to limit the root system of the weed to about two-thirds of its amount when the barley is not present.

Thick seeding of barley, therefore, is very effective in reducing the capacity of *Holcus* as a competitor for a limited amount of root space.

We have already seen (see p. 257) that barley and *H. mollis* are almost equally able to take up nitrogen when the latter is present in ample supply, so that it is of interest to see how the nitrogen recovered is partitioned between the plants when, as in the present case, the amount of the weed remains constant and the thickness of the barley is increased. The results in the three cases already considered are given in Table 9.

Where the barley gets a good start on the weed, and it is thickly enough sown, it is able to capture almost the whole of the nitrogen that is recovered and up to a rate of six plants per pot (2·4 l. root space per plant) the proportion of the recovered nitrogen which is found in the barley varies very closely with the thickness of planting. Where the barley and the weed start level, being sown or planted at the same time, the competition for nitrogen is more even, but with the thickest sowing

of the barley the *Holcus* is still able to get nearly half the nitrogen contained in the combined growth. Where the *Holcus* has already occupied the ground and is already in possession, a single barley plant is only able to get 1 % of the nitrogen recovered. Even here, however, the thickly sown barley, though still poor, has been able to capture 26 % of the nitrogen recovered in the combined growth.

TABLE 9. *Partition of nitrogen taken up in mixed cultures of barley and* Holcus mollis

| No. of plants of | | Mg. nitrogen in barley (whole plants including roots) | Mg. nitrogen in *Holcus* (whole plants) | Percentage N recovered in | |
|---|---|---|---|---|---|
| Barley | *Holcus* | | | Barley | *Holcus* |
| A. Barley sown 9 days before *Holcus* (nitrogen added 1250 mg. per pot) | | | | | |
| 1 | 6 | 666 | 551 | 54 | 46 |
| 2 | 6 | 932 | 386 | 70 | 30 |
| 4 | 6 | 1058 | 293 | 78 | 22 |
| 6 | 6 | 1320 | 106 | 92 | 8 |
| 8 | 6 | 1305 | 108 | 92 | 8 |
| B. Barley sown and *Holcus* planted at same time (nitrogen added 1250 mg. per pot) | | | | | |
| 1 | 6 | 336 | 1161 | 22 | 78 |
| 2 | 6 | 405 | 1115 | 26 | 74 |
| 4 | 6 | 551 | 927 | 37 | 63 |
| 6 | 6 | 789 | 621 | 56 | 44 |
| 8 | 6 | 775 | 689 | 53 | 47 |
| C. Barley sown on land containing 1 year's *Holcus* roots (nitrogen added 1750 mg. per pot) | | | | | |
| 1 | 6 | 23 | 2142 | 1 | 99 |
| 2 | 6 | 46 | 1978 | 2 | 98 |
| 4 | 6 | 145 | 1699 | 8 | 92 |
| 6 | 6 | 229 | 1542 | 13 | 87 |
| 8 | 6 | 458 | 1313 | 26 | 74 |

## II. *Barley plant constant: weed infestation varying*

In the case just considered we studied the result of increasing the density of the barley crop on the development of weed and barley. We now have to examine the effect of increasing the number of weed units on the vigour and development of a sparse barley crop. In order to do this, a fixed number of two barley plants per pot were sown, thus allowing 7·1 l. of root space per plant. The number of *Holcus* plants per pot varied from none to eight, that is to say, from a pot completely free from the weed to a very thick occurrence of *H. mollis*. As in the last case, we have three conditions, namely, (A) when the barley was sown a few days before the *Holcus* was planted, (B) where the two were put in the same day, and (C) where the proper number of *Holcus* plants were already established in the soil. The results are shown in Table 10.

When the barley gets a good start over *Holcus*, as in § A, and with a constant number of plants of barley (forming a thin crop), the weed has little chance against the crop itself. The latter is hardly reduced in yield even when the number of *Holcus* plants is four times as great as those of barley. The maximum amount of

TABLE 10. *Yield (g.) of barley and* Holcus *with varying amounts of* Holcus

| No. of plants per pot | | Maximum no. of barley shoots per plant | Yield of barley per pot | | Yield of total produce per pot | | Yield of roots per pot | |
|---|---|---|---|---|---|---|---|---|
| Barley | *Holcus* | | Grain | Total produce | *Holcus* (above ground) | Barley and *Holcus* together | Barley | *Holcus* |
| | | | A. Barley sown 9 days before *Holcus* | | | | | |
| 2 | 0 | 35 | 41·2 | 102·9 | — | 102·9 | ? | — |
| 2 | 1 | 30 | 34·9 | 95·5 | 7·8 | 103·3 | 3·8 | 6·6 |
| 0 | 1 | — | — | — | 36·0 | 36·0 | — | 31·5 |
| 2 | 2 | 31 | 37·9 | 95·9 | 22·8 | 118·7 | ? | ? |
| 0 | 2 | — | — | — | 31·8 | 31·8 | — | 42·8 |
| 2 | 4 | 25 | 29·2 | 79·1 | 38·3 | 117·4 | 3·1 | 33·2 |
| 0 | 4 | — | — | — | 51·9 | 51·9 | — | 78·4 |
| 2 | 6 | 26 | 33·3 | 84·9 | 31·3 | 116·2 | 3·4 | 24·3 |
| 0 | 6 | — | — | — | 58·4 | 58·4 | — | 73·9 |
| 2 | 8 | 26 | 38·8 | 94·6 | 22·0 | 116·6 | 3·8 | 15·1 |
| 0 | 8 | — | — | — | 74·8 | 74·8 | — | 101·1 |
| | | | B. Barley sown and *Holcus* planted at the same time | | | | | |
| 2 | 0 | 26 | 69·8 | 156·5 | — | 156·5 | 13·0 | — |
| 2 | 1 | 23 | 58·4 | 133·2 | 26·3 | 159·5 | 11·0 | 26·0 |
| 0 | 1 | — | — | — | 93·6 | 93·6 | — | 73·0 |
| 2 | 2 | 22 | 51·3 | 116·1 | 43·1 | 159·2 | 9·6 | 33·9 |
| 0 | 2 | — | — | — | 107·7 | 107·7 | — | 83·3 |
| 2 | 4 | 17 | 35·6 | 82·0 | 67·0 | 149·0 | 6·8 | 52·4 |
| 0 | 4 | - | — | — | 127·1 | 127·1 | — | 88·1 |
| 2 | 6 | 15 | 32·4 | 73·2 | 64·7 | 137·9 | 6·1 | 70·3 |
| 0 | 6 | — | — | — | 113·6 | 113·6 | — | 87·3 |
| 2 | 8 | 16 | 26·1 | 60·8 | 70·5 | 131·3 | 5·0 | 85·9 |
| 0 | 8 | — | — | — | 101·6 | 101·6 | — | 111·6 |
| | | | C. Barley sown on land containing 1 year's *Holcus* roots | | | | | |
| 2 | 0 | 20 | 66·1 | 151·1 | — | 151·1 | 11·3 | — |
| 2 | 1 | 3 | 6·2 | 12·6 | 142·1 | 154·7 | 0·9 | 107·8 |
| 0 | 1 | — | — | — | 144·0 | 144·0 | — | 101·0 |
| 2 | 2 | 6 | 19·0 | 42·4 | 107·8 | 150·2 | 3·2 | 70·8 |
| 0 | 2 | — | — | — | 138·2 | 138·2 | — | 99·9 |
| 2 | 4 | 3 | 4·5 | 8·7 | 159·6 | 168·3 | 0·7 | 101·4 |
| 0 | 4 | — | — | — | 147·2 | 147·2 | — | 150·3 |
| 2 | 6 | 2 | 1·3 | 3·5 | 177·3 | 180·8 | 0·3 | 92·7 |
| 0 | 6 | — | — | — | 163·6 | 163·6 | — | 133·0 |
| 2 | 8 | 2 | 3·6 | 7·0 | 135·0 | 142·0 | 0·5 | 92·6 |
| 0 | 8 | — | — | — | 148·1 | 148·1 | — | 100·2 |

*Holcus* is reached when there were four plants of the weed to two of barley. When, on the other hand, the *Holcus* starts at the same time as the barley and thus competes on equal terms, the effect on the barley is much more substantial. Table 11 shows how this effect increases with the proportion of weed to barley.

The increase in the *Holcus* growth is about the same as the falling off in the barley, showing that the two are almost completely complementary.

TABLE 11. *Effect of increasing proportions of* Holcus *to barley on yield of either, when planted at the same time*

| No. of plants per pot | | Wt. of total produce of barley (including roots) (g.) | Percentage of barley crop without *Holcus* | Wt. of *Holcus*, including roots (g.) | Percentage increase over wt. with one *Holcus* plant |
|---|---|---|---|---|---|
| Barley | *Holcus* | | | | |
| 2 | 0 | 169·5 | — | — | — |
| 2 | 1 | 144·2 | 85 | 52·3 | 100 |
| 2 | 2 | 125·7 | 74 | 77·0 | 147 |
| 2 | 4 | 88·8 | 52 | 119·4 | 228 |
| 2 | 6 | 79·3 | 47 | 135·0 | 258 |
| 2 | 8 | 65·8 | 39 | 156·4 | 299 |

One of the most interesting results shown by these figures is that the presence of two barley plants in a root space of 14·2 l. is able to reduce the growth of one *Holcus* plant to 33% of that when it is grown alone, though there is plenty of space for the roots of both *Holcus* and barley and plenty of nutriment for both. Even when the number of *Holcus* plants is four times as great as that of the barley, the latter is still able to reduce the growth of the weed to 73% of that when it is grown alone.

When *Holcus* has already occupied the soil, as in § C of Table 10, and the barley has to fight against an already established competitor, the results are quite different. After being in the soil for a year, it must be noticed, the *Holcus* roots have already occupied the whole pot of soil, and though there is enough nitrogen present for both the barley and the weed, the barley gets little chance. Whatever the thickness with which the barley is planted, the result is almost the same. The crop obtained was on the average less than 10% of that recovered when the barley was grown alone, and the presence of the barley made no difference to the amount of *Holcus* that grew either above or below the surface of the soil. In fact, once the twitch has thoroughly occupied the soil, it is little affected by the presence of a normally sown barley crop, and is able to suppress the latter almost entirely.

The practical effect of these results seems to be that, provided there is no growing *Holcus* in the ground when a barley crop is sown and the latter can be given a good start on the twitch, and if it can be kept under during the early growth of the barley crop, the latter will be able very largely to suppress the weed. If, on the other hand, it gets a fair start with the barley, it may be able to reduce the latter to from 85 to 45% of its normal weight, according to the thickness of the *Holcus* in the ground. If the weed is thoroughly established in the soil, the presence of sown barley plants will hardly affect its growth.

The way in which the available nitrogen absorbed by the plants distributes itself between barley and *Holcus* when the former is in constant amount and the latter increases in density of planting may be seen from the figures given in Table 12.

TABLE 12. *Partition of nitrogen taken up in mixed cultures of barley and* Holcus mollis, *with increasing density of* Holcus mollis

| No. of plants of | | Amount of nitrogen in barley (whole plants including roots) (mg.) | Amount of nitrogen in *Holcus* (including roots) (mg.) | Percentage N recovered in | |
|---|---|---|---|---|---|
| Barley | *Holcus* | | | Barley | *Holcus* |
| A. Barley sown 9 days before *Holcus* (nitrogen added 1250 mg. per pot) | | | | | |
| 2 | 0 | 1045 | — | 100 | — |
| 2 | 1 | 923 | 154 | 85 | 15 |
| 2 | 2 | 901 | 336 | 73 | 27 |
| 2 | 4 | 787 | 551 | 59 | 41 |
| 2 | 6 | 901 | 477 | 65 | 35 |
| 2 | 8 | 1029 | 397 | 72 | 28 |
| B. Barley sown and *Holcus* planted at same time (nitrogen added 1250 mg. per pot) | | | | | |
| 2 | 0 | 1198 | — | 100 | — |
| 2 | 1 | 948 | 298 | 76 | 24 |
| 2 | 2 | 802 | 435 | 64 | 36 |
| 2 | 4 | 574 | 677 | 46 | 54 |
| 2 | 6 | 497 | 827 | 38 | 62 |
| 2 | 8 | 380 | 885 | 29 | 71 |
| C. Barley sown on land containing 1 year's *Holcus* roots (nitrogen added 1750 mg. per pot) | | | | | |
| 2 | 0 | 1301 | — | 100 | — |
| 2 | 1 | 91 | 1652 | 5 | 95 |
| 2 | 2 | 317 | 1272 | 20 | 80 |
| 2 | 4 | 66 | 1734 | 4 | 96 |
| 2 | 6 | 28 | 1871 | 1½ | 97½ |
| 2 | 8 | 59 | 1573 | 4 | 96 |

When the barley gets a good start before the *Holcus*, it is always able to get over 60% of the nitrogen taken by the two plants together, however thick the *Holcus* plants may be: when the two start level, the relative amounts of nitrogen depend on the thickness of the weed, and with a really dense plant (eight units per pot) the latter is able to get nearly 75% of that found in the two competitors: when the *Holcus* is already well established, it takes very nearly all that is recovered in the two plants together and the original number of units of *Holcus* affects the results very little indeed.

### ROOT INTERACTIONS BETWEEN BARLEY AND *HOLCUS MOLLIS*

It has recently been suggested that there are interactions between a number of species of grasses which cannot be accounted for on the basis of a differential response to light, temperature, moisture, manuring, and management, and it has been interesting to see what indication the present work shows in connexion with this question. While Ahlgren & Aamodt (1939) suggest that there is a good deal of evidence of such interactions, more recently Donald (1946) was able to find no substantial indications in this direction. In the present case, an examination of the combined roots does not suggest any repulsion or otherwise between the roots of the two plants under study. They were simply combined in a thick network of root fibres.

But, on the other hand, the effect of a few plants of barley on the amount of *Holcus* growth as shown in Tables 6 and 10 is very striking. Two cases may be detailed, namely (1) when two barley plants occur with one *Holcus* plant per pot, and (2) where one barley plant occurs with six *Holcus* plants. In neither of these cases is there any question of actual competition for root space, and the results may be taken as an expression of the effect of one plant on the other where the needs of both are amply satisfied. The summarized results are shown in Table 13, those marked 'A' being taken where the barley was sown before the *Holcus* was planted (in 1944), and 'B' where they were put in at the same time (in 1945).

TABLE 13. *Interactions between barley and* Holcus mollis

| | Total yield above ground | |
|---|---|---|
| | A | B |
| I. Effect of two barley plants and one *Holcus* plant per pot | | |
| (*a*) *Holcus* grown alone (g.) | 36·0 | 93·6 |
| (*b*) *Holcus* grown with barley (g.) | 7·8 | 26·3 |
| Reduction of *b* on *a* (%) | 78 | 72 |
| (*a*) Barley grown alone (g.) | 102·9 | 156·5 |
| (*b*) Barley grown with *Holcus* (g.) | 95·5 | 133·2 |
| Reduction of *b* on *a* (%) | 7 | 15 |
| II. Effect of one barley plant and six *Holcus* plants per pot | | |
| (*a*) *Holcus* grown alone (g.) | 58·4 | 113·6 |
| (*b*) *Holcus* grown with barley (g.) | 31·4 | 83·5 |
| Reduction of *b* on *a* (%) | 46 | 26 |
| (*a*) Barley grown alone (g.) | 112·4 | 144·5 |
| (*b*) Barley grown with *Holcus* (g.) | 66·4 | 47·6 |
| Reduction of *b* on *a* (%) | 41 | 67 |

There seems no doubt of the effect of the one plant on the other even though they are grown in presence of ample supplies of water, plant food, etc., and under conditions where no competition for root space can occur. The harmful effect of the barley on *Holcus* seems on the whole to be greater than that of *Holcus* on the barley.

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(*Received* 14 *October* 1946)

Fig. 2. Roots of barley and *Holcus mollis* growing together, showing the complete intermingling of the roots. A, *Holcus mollis* plant; B, barley plant.

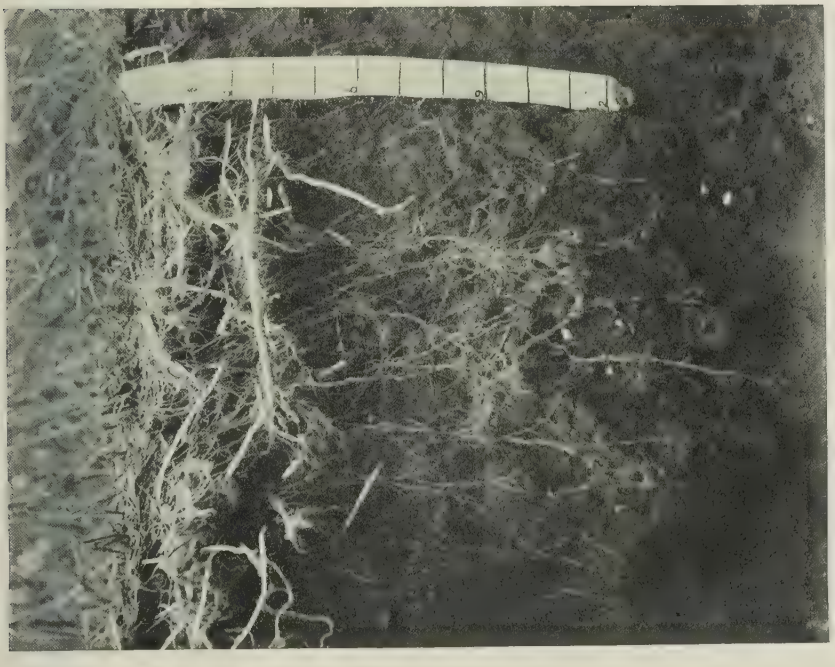

Fig. 1. Root and rhizome growth of *Holcus mollis, in situ,* after 10 months on acid soil at Woburn.

# THE PARASITISM OF *STRIGA HERMONTHICA* BENTH. ON LEGUMINOUS PLANTS

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(With 2 Text-figures)

Pot experiments at the Gezira Research Farm showed that *Striga hermonthica* Benth. is not confined to the Gramineae but can also parasitize groundnuts, cowpea, dolichos bean and soya bean, causing a loss in yield in all but the last-named. The *Striga* plants developing on the roots of these hosts were small, apparently ill-nourished and did not grow more than 1 cm. above the ground, in contrast to the vigorous, flowering *Striga* plants that develop on sorghum. The greatest reduction in yield in these experiments was in dolichos bean, where the aerial growth was reduced 60%; no significant reduction in root weight was found in groundnuts, cowpea or soya bean. The last-named plant appeared to be feebly parasitized by *S. hermonthica*. A reduction in total nodule weight, but not in number of nodules, was obtained in groundnuts and cowpea. Parasitized dolichos bean showed a reduction in both the number of nodules and in nodule dry weight; in groundnuts, the later fruit production was seriously reduced.

The use of these leguminous crops to clean *Striga*-infested soil is discussed.

## Introduction

Early workers on the parasitism of *Striga asiatica* (L.) D. Kuntze (= *S. lutea* Lour.) on sorghum have assumed that only root excretions of grasses will induce germination of the *Striga* seed and that only members of the Gramineae can act as host plants (Saunders, 1933). Later, Rose & Lochrie (1941) showed that germination of the seed of this species of *Striga* could be stimulated by excretions from roots of other plants, including members of the Leguminoseae. In a replicated experiment where groundnuts, sugar bean (*Phaseolus vulgaris* L.), sunn hemp and buckwheat were sown previous to a maize crop (which is also attacked by this parasite), these workers showed that 'there was good reason for suspecting that groundnuts, sugar beans, and sunn hemp had actually destroyed much of the *Striga* seed by causing it to germinate' and later die owing to the presumed lack of a host plant. Brown & Edwards (1945) have shown that under certain conditions thiourea and allylthiourea will bring about germination of a small percentage of *Striga asiatica* seed in the absence of the host stimulant. Andrews (1945) showed that the excretions from the roots of leguminous and other non-gramineous plants will induce germination of the seed of *S. hermonthica* Benth. In his experiments no evidence was obtained that the haustoria of this species of *Striga* could parasitize these plants.

In view of these results, pot experiments were started to determine if the sowing of a leguminous crop would rid the soil of *Striga* seed, and permit a successful

sorghum crop to be grown in it. In the course of the experiments it was found that *S. hermonthica* Benth. can parasitize dolichos bean (*Dolichos lablab* L.) and reduce the crop. Preliminary notice of this parasitism has already been given (Andrews, 1946).

The present paper describes experiments made to study in more detail the reactions of certain leguminous crop plants to parasitism by *Striga hermonthica*.

## EXPERIMENTAL

*Experiment* 1. Five seeds of dolichos bean were sown 28 December 1944 in each of thirty 10 in. pots filled with Gezira soil (see Joseph, 1925). In half the pots 0·2 g. *Striga* seed per pot was intimately mixed with the soil. On 4 April 1945 the aerial growth was cut off and dry weighed, the roots were washed, and the number of parasitizing *Striga* plants counted. Those found were all subterranean, small and apparently ill-nourished. Results obtained are given in Table 1. The difference between shoot weights is highly significant ($P=0{\cdot}01$).

TABLE 1. *Records of dolichos bean crop (mean of four plants per pot)*

| | Without *Striga* | With *Striga* |
|---|---|---|
| Height (cm.) | 45 | 22 |
| *Striga* plants on roots | — | 28 |
| Shoots: dry weight (g.) | 14·3 | 8·1 |

*Experiment* 2. Five seeds (later thinned to four) of dolichos bean were sown 20 September 1945 in each of thirty 14 in. pots; in half the pots 0·4 g. *Striga* seed was added per pot. On 8 December 1945 records were made of the dry weights of aerial growth and roots, number and dry weight of the nodules, and number of *Striga* plants on the roots. At this date, above-ground growth of the parasite had started. Results are shown in Tables 2 and 3 and in Fig. 1.

Growth observations started 15 October 1945 (24 days from sowing) by which date the effect of *Striga* attack was already apparent (see Fig. 1). The observations were discontinued on 19 November 1945 when the straggling habit of the bean made further measurements difficult. At this date the mean height of the non-parasitized dolichos plants was $3\frac{1}{2}$ times that of those attacked by *Striga*. The parasitized plants were glaucous grey and apparently undernourished. Table 2 shows the frequency distribution of mean leaflet length after the experiment had continued 10 weeks.

TABLE 2. *Lengths of dolichos bean leaflets in Exp. 2: records made 19 November 1945*

| | % length frequency (cm.) | | | | | | | | Total leaflets (60 plants) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| Without *Striga* | 0·6 | 1·8 | 8·1 | 10·9 | 17·8 | 30·4 | 25·7 | 4·6 | 2320 |
| With *Striga* | 0·3 | 10·3 | 30·7 | 24·7 | 21·4 | 10·4 | 1·7 | 0·2 | 1736 |

The peak frequency of the leaflets of the non-parasitized plants is at 6 cm. while that of the parasitized is at 3 cm.

Exp. 2 was concluded on 8 December 1945, by which date five *Striga* plants distributed over four pots had appeared about 1 cm. above the ground. The records of flowering and of growth of the dolichos plants on 8 December 1945 are assembled in Tables 3 and 4 respectively.

TABLE 3. *Flowering records of dolichos plants* 8 *December* 1945

| | First flower | Pots showing flowers | Total flowering peduncles | Mean height of flowering peduncles (cm.) | Total flowers | Mean total flowers per peduncle |
|---|---|---|---|---|---|---|
| Without *Striga* | 23. xi. 45 | 13 | 42 | 30·3 | 466 | 11·0 |
| With *Striga* | 3. xii. 45 | 7 | 11 | 21·1 | 68 | 6·1 |

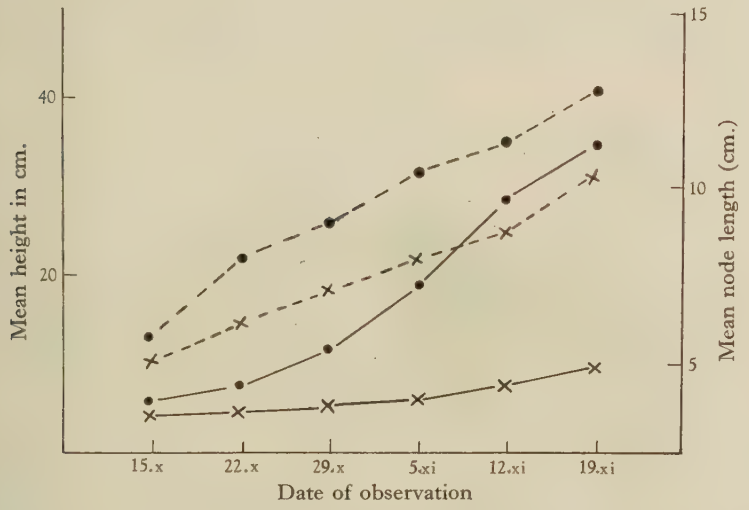


Fig. 1. Mean height and nodes of dolichos plants.

●——● Mean height non-parasitized plants. × —— × Mean height parasitized plants.
●- - - -● Mean node length non-parasitized plants.
× - - - - × Mean node length parasitized plants.

TABLE 4. *Growth records of dolichos plants* 8 *December* 1945 (*means per pot of four plants*)

| | Dry wt. (g.) | | | Total nodules |
|---|---|---|---|---|
| | Roots | Leaves | Nodules | |
| Without *Striga* | 9·3 | 36·6 | 2·0 | 87·8 |
| With *Striga* | 5·3 | 13·8 | 1·5 | 59·7 |

(Difference significant at $P = 0{\cdot}05$.)

Table 4 shows that the parasitism of *S. hermonthica* on dolichos bean causes a significant loss of weight of all parts of the host plant. It is interesting to note the decrease in the number of nodules; this may be connected with the decrease in weight of the parasitized roots.

The counts of *Striga* plants found at the conclusion of Exp. 2 showed that the mean number per infected pot was 455. One plant measured 7·1 cm., but the average length was only 2·9 cm. The parasites were thus very small by comparison with sorghum-infecting *Striga* plants, which make luxuriant aerial growth in pots in a similar period. The subterranean plants on the dolichos bean roots were a pale translucent pink, but although small and undernourished they were similar in structure to the plants infecting sorghum.

*Experiment* 3. In this experiment, six different leguminous plants were tested to discover if *S. hermonthica* Benth. could parasitize them and cause a loss in crop. It had been previously shown that root excretions from all these species could induce germination of *Striga* seed. Dolichos bean was included as a control plant.

*Treatments*

A. Soil untreated.
B. Soil artificially infested with *Striga* seed.

Leguminous plants

(1) Groundnut—local variety, *Arachis hypogea* L.
(2) Sunn hemp—Indian variety, *Crotalaria juncea* L.
(3) Soya bean—black seeded variety, *Glycine max* (L.) Merr.
(4) Cowpea—brown seed, local variety, *Vigna unguiculata* Walp.
(5) Dolichos bean—local variety, *Dolichos lablab* L.
(6) Lucerne—local variety, *Medicago sativa* L.

Details of pots, soil, etc., were as in Exp. 2.

Seven replicates of each crop were made in treatments A and B. With the exception of lucerne, eight seeds were sown per pot, thinned to four plants of equal size after 14 days. The lucerne pots contained on an average fifty-three plants. On 19 July 1946 all pots were sown and watered equally. The germination of the soya bean seed was very poor and it was resown on 1 August 1946. On 1 October 1946 watering ceased. By this date several pots were showing *Striga* plants appearing above the soil, though the maximum height was only about 1 cm.

The aerial growth of the host plants was cut off and dry weighed, the roots washed out and records made of the number and length of the *Striga* plants attached, as well as the other details recorded for Exp. 2.

No *Striga* plants were found parasitizing the sunn hemp and lucerne: these have therefore been omitted from the results given in Tables 5–7.

TABLE 5. *Details of* Striga *plants* (*totals for seven pots*)

| Plant | Total *Striga* above soil level | Total *Striga* on roots |
|---|---|---|
| Groundnut | 25 | 471 |
| Soya bean | 1 | 15 |
| Cowpea | 8 | 677 |
| Dolichos bean | 11 | 707 |

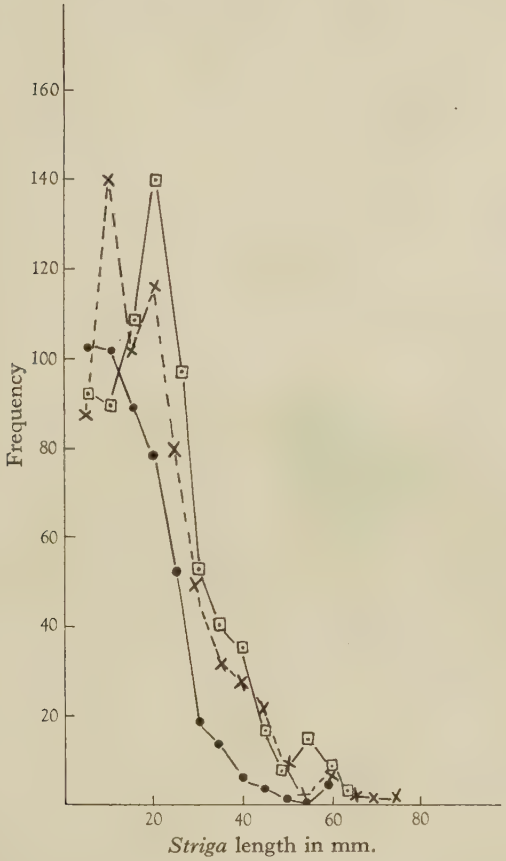


Fig. 2. Length frequency of *Striga* plants on roots of leguminous plants.

●——● Groundnuts; × — — — × Cowpea; ⊡——⊡ Dolichos bean.

*Results.* Fig. 2 shows the length frequency of the *Striga* plants attached to the roots. The maximum for groundnuts is at the 5 mm. level, that for cowpea at the 10 mm. level and that for dolichos bean at the 20 mm. level. This suggests

that the *Striga* plants on dolichos bean roots were more successful than those on the roots of the other two plants.

Soya bean has been omitted from Fig. 2 since a total of only fifteen *Striga* plants occurred on its roots. The greatest number (five) occurred at the 15 mm. level, while one reached 60 mm. The mean values per plant are given in Table 6 to afford a proper comparison, since variation from three to four occurred in the number of plants per pot that survived to the end of the experiment. In the statistical analysis of the results, the values from the unit plant per pot were used.

TABLE 6. *Nodule formation, aerial growth, and root data (mean values per plant)*

| Treatment | A. Without *Striga* | | | | B. With *Striga* | | | |
|---|---|---|---|---|---|---|---|---|
| Plant | No. of nodules | Nodules dry wt. (g.) | Aerial dry wt. (g.) | Roots dry wt. (g.) | No. of nodules | Nodules dry wt. (g.) | Aerial dry wt. (g.) | Roots dry wt. (g.) |
| Groundnut | 94·00 *N* | 0·27* | 4·40† | 1·45 *N* | 91·00 | 0·12 | 3·07 | 1·51 |
| Soya bean | 5·71 *N* | 0·06 *N* | 4·29 *N* | 1·78 *N* | 3·85 | 0·05 | 5·02 | 1·90 |
| Cowpea | 34·57 *N* | 0·49* | 5·77* | 2·45 *N* | 29·42 | 0·29 | 3·07 | 2·36 |
| Dolichos bean | 23·85* | 0·57* | 8·73* | 2·86* | 10·28 | 0·32 | 2·77 | 1·79 |

* Difference between means of A and B significant at $P=0{\cdot}01$.
† Difference between means of A and B significant at $P=0{\cdot}05$.
*N* Difference between means of A and B *not* significant at $P=0{\cdot}05$.

The parasite has caused a loss in crop in groundnuts and cowpea as well as in dolichos bean. Soya bean does not in this experiment appear to be affected, but this plant was sown later than the other three leguminous plants, and it is possible that had the experiment continued for a longer period, the yield of soya bean might also have been affected. It is noteworthy that only fifteen *Striga* plants had attached themselves to the roots of soya bean and it would appear that soya bean interposes a definite resistance to penetration by the haustoria of the parasite.

Little significance can be attached to the number of *Striga* plants appearing above ground since this must undoubtedly depend to a large extent on the depth below soil surface at which parasitism took place. It is noteworthy that in no case did the *Striga* plants reach more than 1 cm. above ground, though if the parasitized plant had been a sorghum, the *Striga* would have flowered by the end of the experiment. It is known that if a sorghum plant is very heavily attacked by *S. hermonthica* a very small proportion of the parasites appear above ground and flower, owing presumably to the nutrients available from the roots of the host plant not being sufficient to bring a large number of *Striga* plants above ground and to maturity. This may be the cause of the poor growth of *Striga* on the leguminous plants but it is more probable that the parasites do not extract the necessary nutrients from the leguminous roots with the ease that they do from sorghum roots. In only one case out of a number of pot experiments has the author seen flowering *Striga* plants on the roots of dolichos bean. This has never been noticed in the field.

It has been shown in the case of sorghum that the number of plants parasitizing the roots of the host plant is dependent on the latter's root development (Andrews, 1945). In dolichos bean, cowpea and groundnuts, the number of *Striga* plants attached to the roots varies with the root weight, the greater root weight having the greater number of parasites. The appearance of the subterranean plants of *S. hermonthica* on these crops was similar to those described on the roots of dolichos bean in Exp. 2.

In comparison with the other legumes tested, the most affected plant is dolichos bean which alone shows when parasitized a significant reduction in root weight and number of nodules. It seems clear that in groundnut and cowpea the nodules, though not differing in number, were either smaller in size in the parasitized plants or contained less solid matter. Only in soya bean is there no significant reduction in the weight of the nodules.

There was no significant difference in the flower production of the parasitized and non-parasitized plants. Dolichos bean produced no flowers. Crowther & Barlow (1943) have shown that dolichos bean on the Gezira Research Farm will flower only during a period of short days commencing roughly from November. This experiment ended before that period arrived.

Fruit production was negligible except in groundnut (Table 7). In this plant there is no significant difference between the number of ripe fruit, but there is

TABLE 7. *Details of groundnut fruit production*

| Treatment ... | A (without *Striga*) | | B (with *Striga*) | |
|---|---|---|---|---|
| Details ... | Ripe fruit | Unripe fruit | Ripe fruit | Unripe fruit |
| Mean no. per plant | 2·97 *N* | 3·14* | 2·70 | 1·01 |
| Mean wt. per fruit (g.) | 0·21* | 0·04* | 0·15 | 0·02† |

* Difference of means significant at $P=0{\cdot}01$.
† Only five pots out of seven contained unripe fruit.
*N* Difference of means *not* significant at $P=0{\cdot}05$.

No fruits were produced on soya bean or dolichos bean and extremely few on cowpea by the end of this experiment.

between the mean weight per ripe fruit and also between the number and mean unit weight of the unripe fruit. It appears that parasitism of this crop reduces seriously the later production of fruit and thus lowers the final yield.

## DISCUSSION

It is evident from the above experiments that *S. hermonthica* can parasitize host plants other than members of the Gramineae and cause a reduction in their yield. It may be noted that although the extract from the roots of sunn hemp and lucerne can germinate the seed of *Striga* the latter could not parasitize these plants under the conditions of these experiments.

It is probable that the degree of resistance to parasitism of the crops under test will differ with variety, particularly in soya beans as is reputed to be the case among cultivated sorghums (Crowther, 1942).

It will be noted that the dolichos bean plants were sown at different times of the year in the three experiments, viz. in December, September and July. The plants in the three experiments would therefore be growing under different climatic conditions. The duration of the three experiments differed also, the first experiment lasting for 97 days, the second for 79 days and the third for 76 days. The relative effects of the parasitism are therefore likely to differ somewhat in each experiment. In the first experiment the shoot yield was decreased by 43·3%: in the second experiment, the leaf and stem yield was reduced by 62·4% and the roots by 42·5%, and in the third experiment the aerial growth was decreased by 68·3% and the roots by 37·4%. In the last two experiments pots of the same size were used and there is a similarity in the loss caused by the parasite.

Little is known about the process by which the parasite obtains its food from the roots of its host plant, or of the details of the nutrients which it extracts. Stephens (1912) working with *S. asiatica* (L.) D. Kuntze on maize says: 'The whole question of the nutrition of this parasite especially during the underground portion of its life-history is obscure.... Until the subaerial shoot is formed the plant thus apparently has to depend for its supply of assimilated food on the reserve stores passed over from the phloem to the xylem of the host and on what the haustoria may absorb from the cells dissolved by its ferments. Many microchemical tests have been made to find out what foodstuffs are transferred from host to parasite but without result.' Saunders (1933), working also on *S. asiatica*, states that 'assimilated foods are taken from the host plant in liberal quantity, for while apparently little or no storage takes place in the haustorium itself, large quantities of starch are stored in the young subterranean shoot'. He further goes on to say that 'the haustorium contains relatively large amounts of phosphate and small quantities of nitrate nitrogen and potassium salts'. In appearance, a parasitized crop seems to be suffering from shortage of nitrogen, and it is known that the addition of a nitrogenous fertilizer to the land increases the yield of sorghum sown in soil containing *Striga* seed (Crowther, 1942). It seems possible that with the addition of nitrogen to the soil, more is available in the host roots for the development of the host plant after the parasite has taken its quota. There is however no direct evidence in support of this hypothesis. The nitrogenous fertilizer may cause a change in the sorghum root, rendering it less susceptible or may interfere with normal germination of the *Striga* seed. The ultimate effect of the parasitism on any particular plant will be caused by the resultant of the number of *Striga* parasites on the host root, the size of the parasites and the efficiency with which they extract nutrients from the host root. The latter quantity is difficult to estimate, obviously varies between different host plants and may differ between roots of the same plant.

The ratio of the number of nodules to the root weight is not significantly different in the parasitized and non-parasitized plants in both Exps. 2 and 3, nor is there

a significant regression coefficient between the number of nodules and root weight in any of the parasitized and non-parasitized plants under discussion. In groundnuts and cowpea, though the numbers of nodules of parasitized and non-parasitized plants do not differ significantly there is a significant reduction in the weight of the nodules. The cause of this is obscure.

It is clear from these experiments that the sowing of groundnuts, cowpea and dolichos bean, in a *Striga* infested soil must rid that soil of a large proportion of its *Striga* seed. The fact that in no experiment did the *Striga* plants reach more than 1 cm. above soil level suggests that the nourishment supplied by the host plants is not sufficient to bring the *Striga* plants to maturity. Thus the germinated *Striga* seed in the soil would not be replaced by fresh seed and a cleaning of the soil would result, though loss in the yield of the host crop would inevitably occur. These leguminous plants would therefore act as a trap crop, and with repeated sowings should restore *Striga*-infested land to a suitable condition for raising a successful crop of sorghum. In sunn hemp and lucerne which do not undergo parasitism although they can apparently germinate the *Striga* seed, it is not known if the quantity of germination bears any relation to that caused by the parasitized plants dealt with in this paper. It may well be that the germinating power of the excretions from roots of sunn hemp and lucerne is feeble and that although a loss of crop results among the parasitized leguminous plants, the quantity of germination of *Striga* seed caused by the latter may make it advantageous to sow them rather than the non-parasitized sunn hemp and lucerne.

I have pleasure in acknowledging the assistance I have received from Zein Eff. Abdel Nabi, Senior Technical Assistant, and other members of the staff in the collection of the data.

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(*Received* 26 *November* 1946)

# THE ROLE OF ANTIBIOTICS IN THE DECOMPOSITION OF SAWDUST

## I. INHIBITION OF THE GROWTH OF CELLULOSE-DECOMPOSING BACTERIA

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It has been shown that 'deal' sawdust contains substances which inhibit the growth of cellulose-decomposing bacteria of the genera *Sporocytophaga* and *Cellulomonas*, the former being the more sensitive. The substances can be extracted with water or a mildly alkaline solution of inorganic salts, the latter being rather more effective. The extracted material is acidic but still exhibits activity even after neutralization, especially towards *Sporocytophaga*. Sawdust which has been extracted with water or the alkaline solution, or neutralized by admixture with calcium carbonate, no longer prevents the growth of *Cellulomonas* on added filter paper cellulose, although there is a delay before the attack becomes evident, but *Sporocytophaga* is still completely inhibited under all three conditions.

### Introduction

In view of its abundance, cheapness, and ability to absorb between four and six times its own weight of water, sawdust would be a good material to serve as litter for animals in place of straw, except that it is very resistant to attack by micro-organisms and cannot readily be converted after use into manure suitable for application to the land. The value of sawdust as a means of increasing the humus content of the soil has been investigated in the past, and claims both in favour of and against its use have been made (see Putnam, 1916; Barthel & Bengtsson, 1926; Newton & Daniloff, 1927; Motte, 1931; Nostitz, 1937). When added directly to soil, sawdust decomposes only slowly. This is in striking contrast to the readiness with which other cellulosic materials such as straw, hay and leaves are attacked, and is surprising in view of the abundance of cellulose-decomposing micro-organisms in the soil. Sawdust, however, is not simply cellulose but contains *inter alia* a considerable proportion of lignin. This material is the most resistant of the major plant constituents to microbial attack, and its presence has a marked influence on the ease with which any plant material can be decomposed. It has been suggested that lignin, which is deposited in both the cell wall and the middle lamella, acts by interposing a mechanical barrier which renders the more easily decomposable substances less accessible to attack (Norman, 1937), but in addition a bacteriostatic function has been claimed for isolated acid lignin (Boruff & Buswell, 1934). It is now clearly recognized that antibiotics are present in many plant tissues, and it is noteworthy that the heart-wood of pine contains an antibiotic, pinosylvine (*trans*-3:5-dioxystilbene), which protects it after death against attack by fungi and insects (Frykholm, 1945) and which exhibits antibacterial activity.

It seemed therefore that the slow rate of decomposition of sawdust might be due at least in part to the presence of antibiotics, and it was decided to investigate this matter. The present paper is concerned with the aerobic bacteria which attack cellulose, and the cellulose-decomposing fungi will be dealt with in a subsequent communication.

## Experimental

### (*a*) *Materials*

No doubt the woods of different trees vary considerably in their content of antibiotics, but as it is known that coniferous woods do contain substances of a phenolic type which would on general grounds be expected to show antibacterial activity, it was decided to use 'deal' sawdust derived from a variety of white coniferous woods. It was obtained from a carpenter's shop and consisted entirely of clean dry particles of which only the portion passing through a 2 mm. sieve was used.

Two species of cellulose-decomposing bacteria were tested, namely *Sporocytophaga myxococcoides* (Stanier), more usually known as *Spirochaeta cytophaga* (Hutchinson & Clayton), and an unidentified member of the genus *Cellulomonas*. For inoculation purposes, cultures of these bacteria were grown in the medium devised by Dubos (1928), which has a *p*H of 7·5 and in which filter paper forms the source of carbon. *Cellulomonas* cultures were grown at 25° C. for 3 days, and *Sporocytophaga* cultures at 30° C. for 3–5 days, the cultures being well shaken before use to dislodge the organisms from the paper and provide as even a suspension of cells as possible. The paper fibres were allowed to settle before the suspension was used, 0·1 ml. being the standard quantity employed for an inoculation. This amount, when added to a tube containing 5 ml. of Dubos's medium, produced a very evident decomposition of the filter paper after only 2 or 3 days' incubation.

### (*b*) *The survival of cellulose-decomposing bacteria in the presence of sawdust*

To obtain information on the antibacterial substances occurring in sawdust, the cellulose-decomposing bacteria were added to moist sawdust in the presence of an adequate supply of available nitrogen and mineral salts, and an attempt made to follow the progress of the cultures by counting the bacteria at weekly intervals. 1 g. portions of sawdust were placed in 100 ml. conical flasks and 10 ml. of Dubos's basal solution (the medium without filter paper) added to each, this quantity being sufficient to saturate the sawdust and leave free liquid in the flasks. Two series were prepared, one inoculated with 0·1 ml. of *Cellulomonas* suspension per flask, and the other with 0·1 ml. of *Sporocytophaga* suspension. The contents of the flasks were shaken and finally left so that the sawdust was in a heap at one side of each flask and projecting above the surface of the liquid, thus providing the correct physical conditions for the development of these bacteria. One flask of each series was set aside for an immediate count of the cellulose-decomposing bacteria present,

and the remainder were placed in the incubator at 25° C. for the *Cellulomonas* series and at 30° C. for the *Sporocytophaga* series.

It is not possible to count cellulose-decomposing bacteria by the usual plating technique, so the dilution method was adopted. A series of dilutions of the contents of a flask were made in sterile tap water, progressing in steps of ten. From each dilution ten replicate 1 ml. samples were taken, each being placed in a tube of Dubos's medium containing filter paper. The tubes were incubated at the appropriate temperature and examined after 7 days for signs of growth. The numbers of positive and negative cultures from each dilution were noted and from the tables of Halvorson & Ziegler (1936) the probable number of bacteria in the original culture was obtained. In this way it was found that the number of bacteria added to each flask in the 0·1 ml. of inoculum greatly exceeded 10,000. Counts were made on a flask of each series immediately after inoculation, and again after 7 and 14 days' incubation.

In no case was even a single positive dilution culture obtained. This indicated that not only had the bacteria failed to grow even slowly in the flask cultures, but there appeared to have been a rapid diminution in numbers after only a few minutes' contact with the sawdust. Adsorption on the sawdust particles could have been the reason for the failure to recover bacteria added shortly before the count was performed, but could hardly have accounted for the negative results from the incubated flasks if there had been even a moderate development during incubation. Failure of this to occur could have been due to the sawdust cellulose being unavailable for mechanical reasons, but this in itself could not have caused a rapid diminution in numbers. The experimental findings could however be accounted for by the presence in the sawdust of antibiotics.

### (*c*) *The presence of antibiotics in sawdust*

To test this point a 1 : 10 extract of sawdust in Dubos's basal medium was prepared, an overnight treatment at 30–40° C. being adopted. The mixture was filtered and the filtrate placed in test-tubes in 5 ml. quantities with a strip of filter paper added to each. After inoculation with either *Cellulomonas* or *Sporocytophaga* suspension the tubes were incubated, but no signs of decomposition of the filter-paper were seen even after 3 weeks, and this strongly indicated that inhibitory substances were present in the sawdust extract. There was however the possibility that during the preparation of the extract the nitrogenous or other constituents of the basal medium might have been adsorbed on to the sawdust, so that the final medium was deficient and unable for that reason to support growth. This was checked by making a 1 : 10 extract overnight at 30–40° C. as before but in distilled water, and mixing the filtered extract in varying proportions with the basal medium. Concentrations of from 50 to 1 % were employed, double strength Dubos's solution being used for the higher concentrations to avoid undue dilution of the inorganic constituents of the final medium. Duplicate tubes of each concentration were

inoculated with each of the organisms and incubated for 3 days. A similar series of tubes was set up using a Dubos's solution extract for comparison, but in this case the undiluted extract was also tested. The results are given in Table 1, from which it is clear that the *Cellulomonas* was inhibited only by the undiluted Dubos's solution extract. With the technique employed the undiluted water extract could not be tested, so it is not possible with this organism to determine whether failure to develop in the undiluted Dubos's solution extract was due to the presence of inhibitors or to the possible lack of nutrients. However, in the case of the *Sporocytophaga* it is clear that the water extract produced some inhibition even when diluted tenfold, and this could not have been due to the lack of nutrients. The presence of inhibitory substances was therefore indicated, and *Sporocytophaga myxococcoides* is evidently considerably more sensitive to these than is the *Cellulomonas* species tested. When incubated for a further 4 days there was no change in the *Cellulomonas* cultures, but growth of the *Sporocytophaga* appeared in the second tube of the 10% water extract as well as in both tubes of the 10% Dubos's solution extract, so the antibiotics in sawdust appear to have a bacteriostatic action at that concentration.

TABLE 1. *The inhibition of* Sporocytophaga myxococcoides *and a* Cellulomonas *species by* 1 : 10 *sawdust extracts*

| | *Sporocytophaga myxococcoides* | | | | *Cellulomonas* species | | | |
|---|---|---|---|---|---|---|---|---|
| | Water extract | | Dubos's solution extract | | Water extract | | Dubos's solution extract | |
| Concentration of extract (%) | (*a*) | (*b*) | (*a*) | (*b*) | (*a*) | (*b*) | (*a*) | (*b*) |
| 100 | − | − | o | o | − | − | o | o |
| 50 | o | o | o | o | + | + | + | + |
| 10 | + | o | o | o | + | + | + | + |
| 1 | + | + | + | + | + | + | + | + |
| Control, no extract | + | + | + | + | + | + | + | + |

+, indicates growth; o, no growth; −, not tested.

The presence of antibiotics active against *Sporocytophaga* having thus been established it seems probable that the inhibition of *Cellulomonas* by the undiluted Dubos's solution extract was also due to antibiotics rather than to the indirect cause suggested above as a possibility.

### (*d*) *The nature of the antibiotics in sawdust*

As shown in Table 1 the Dubos's solution extract appeared to be rather more potent than the water extract. This solution has a *p*H of 7·5, and perhaps the mild alkalinity facilitated the extraction of acidic materials from the sawdust. It is therefore possible that the inhibitions observed might have been due to a change of reaction towards the acid side, since both *Cellulomonas* and *Sporocytophaga* are known to be favoured by neutral or slightly alkaline conditions. Examination of

distilled water and Dubos's solution extracts of sawdust revealed that the former had a *p*H of 5·0 and the latter one of 5·6, so that acidity alone could have accounted for the failure of these organisms to grow in the undiluted Dubos's solution extract. On dilution with an equal volume of Dubos's solution the *p*H of both extracts rose to 7·0 approximately, and thus the inhibition of *Sporocytophaga* which occurred at the 50% concentration could hardly have been due to acidity effects alone and was probably due to some specific inhibitor. The acidity effect must however be countered in any attempt to decompose sawdust itself with the aid of these bacteria, and here it may be mentioned that the *p*H of a 1 : 10 suspension of sawdust in distilled water was as low as 4·3. The difference between this and the figure of 5·0 obtained for the filtered extract indicates that sawdust is of an 'acidoid' nature (Bradfield, 1927).

To reveal whether acidity alone was the cause of the inhibitory action of sawdust extracts or whether specific substances were also present, the activity of a Dubos's solution extract after neutralization to *p*H 7·5 with caustic soda was compared with that of the unneutralized extract. Furthermore, with the object of increasing the potency of the extract, sawdust was treated in this case for 3 days instead of overnight with Dubos's solution, at a temperature of 30° C. The neutralized and unneutralized extracts were mixed in varying proportions with fresh Dubos's solution, a strip of filter paper added, and the tubes inoculated. Each concentration was tested in duplicate, and the results obtained after different periods of incubation are given in Table 2.

TABLE 2. *Comparison of the activity against* Sporocytophaga myxococcoides *and a* Cellulomonas *species of a Dubos's solution extract of sawdust before and after neutralization and with various periods of incubation*

| Concentration of extract (%) | Incubation period: 2 days | | | | 3 days | | | | 7 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E | | EN | | E | | EN | | E | | EN | |
| | *Sporocytophaga myxococcoides* | | | | | | | | | | | |
| 100 | o | o | o | o | o | o | o | o | o | o | o | o |
| 50 | o | o | o | o | o | o | o | o | o | o | o | o |
| 10 | o | o | o | o | o | o | o | o | + | + | + | + |
| 1 | o | tr. | tr. | tr. | + | + | + | + | + | + | + | + |
| Control, no extract | | + | + | | | + | + | | | + | + | |
| | *Cellulomonas* species | | | | | | | | | | | |
| 100 | o | o | o | o | o | o | o | o | o | o | + | + |
| 50 | o | o | o | o | o | o | + | + | + | + | + | + |
| 10 | + | + | + | + | + | + | + | + | + | + | + | + |
| 1 | + | + | + | + | + | + | + | + | + | + | + | + |
| Control, no extract | | + | + | | | + | + | | | + | + | |

E indicates unneutralized extract; EN, neutralized extract. o indicates no growth; +, good growth; tr., a trace.

Treatment of the sawdust with Dubos's solution for 3 days provided an extract which was more potent against the *Cellulomonas* than that obtained by overnight extraction, but the activity towards the *Sporocytophaga* was not enhanced (cf. Table 1). However, the *p*H of the 50% concentration was in this case somewhat lower at 6·5, against that of 7·0 obtained with overnight extraction, so acidity could have accounted for the increased potency against *Cellulomonas*. At a concentration of 10% the *Sporocytophaga* was inhibited for at least 3 days by the unneutralized extract, but as the *p*H in this case was 7·1 the presence of inhibitory substances is indicated. This conclusion is borne out by the results from the neutralized extract with both organisms, since although the *p*H was 7·5 in all cases, marked inhibitory effects were obtained, especially against the *Sporocytophaga*, which was evidently the more sensitive organism. It should be noted that growth was not seen in 3 days in the undiluted neutralized extract with *Cellulomonas*, or in the 10% extract with *Sporocytophaga*, but in both cases the organisms became active in 7 days. The antibiotics in sawdust are evidently bacteriostatic at these concentrations for the respective organisms, but it is possible that at higher concentrations the substances may be bactericidal for the *Sporocytophaga*.

Some experiments were made on the stability of the active substances towards heat. A 3 days Dubos's solution extract was autoclaved at 120° C. for 20 min. both in its original acid condition and after neutralization with excess calcium carbonate. The activity towards the *Sporocytophaga* was retained in the face of both treatments, the results being similar to those given in Table 2 for the unheated extract. Against the *Cellulomonas* however the activity was apparently diminished, since after 3 days' incubation growth had occurred in the presence of 50% of the unneutralized extract whilst autoclaving after neutralization enabled growth to occur in this period even in the undiluted extract (cf. Table 2).

### (*e*) *Attempts to eliminate the antibacterial properties of sawdust*

The work reported above has shown that sawdust contains antibacterial substances in addition to acidic materials and that both are partially or wholly soluble in water or a mildly alkaline solution of inorganic salts. It was accordingly decided to see whether extraction of sawdust with water, or with Dubos's solution, or the addition of a neutralizing agent with or without preliminary extraction, would so diminish the antibiotic activity as to permit the survival and perhaps growth of the cellulose-decomposing bacteria. The ultimate object was to provide, if possible, conditions in which the cellulose of the sawdust could be decomposed, but in view of the fact that such experiments would be prolonged and would involve the chemical estimation of the cellulose, a technique was adopted which would permit the ready detection of active cellulose-decomposing bacteria.

1 g. portions of sawdust which had been treated in various ways were placed in 100 ml. conical flasks each of which contained a strip of filter-paper about 4 × 1 in. arranged so that it extended up the flask wall. Then 10 ml. of Dubos's solution were

added, the flasks inoculated in the usual way and incubated, the expectation being that if the sawdust treatment had been such as to allow the survival of the added bacteria, this should become evident in the course of a week or so through the decomposition of the filter paper. Naturally no conclusions could be drawn about the decomposition of the sawdust cellulose which might still, for physical reasons, be resistant to attack.

Sawdust extracted as above for 3 days with distilled water, as well as with Dubos's solution, was used, unextracted sawdust forming the control. In all three cases separate series were set up in which 1 g. of calcium carbonate was added to each flask to counteract any acidity effect. Weekly examinations were made and the results from the flasks inoculated with the *Cellulomonas* are given in Table 3.

Untreated sawdust proved so inhibitory that no signs of the decomposition of the filter paper were seen even after 3 weeks. Extraction with water alone did not make the conditions suitable, but the mildly alkaline Dubos's solution removed much of the inhibitory material, and signs of attack on the filter-paper were evident in 7 days. At the end of the experiment the *p*H values of the contents of the flasks containing the unextracted, water-extracted and Dubos's-solution-extracted sawdust were respectively 6·3, 6·9 and 7·5. This suggests that the *Cellulomonas* was inhibited mainly but not exclusively by acidity in the sawdust, a conclusion supported also by the fact that when calcium carbonate was added the bacteria survived and attacked the filter-paper in 14 days, even in the presence of unextracted sawdust. The filter-paper was also attacked in 14 days in the presence of water-extracted sawdust plus calcium carbonate, but even in the best circumstances the onset of the attack on the filter-paper was delayed in comparison with that in cultures devoid of sawdust, where vigorous decomposition is often seen in 2 days. This is further evidence for the presence of specific inhibitory substances, and shows that complete removal was not effected by a single extraction even with Dubos's solution.

Table 3. *The effectiveness of various treatments designed to remove or neutralize the* Cellulomonas*-inhibiting properties of sawdust*

| Sawdust treatment | Growth of *Cellulomonas* on added filter-paper Incubation period (days) | | |
|---|---|---|---|
| | 7 | 14 | 21 |
| Untreated | o | o | o |
| Extracted with water | o | o | o |
| Extracted with Dubos's solution | tr. | + | + |
| Unextracted, $CaCO_3$ added | o | + | + |
| Extracted with water, $CaCO_3$ added | o | + | + |
| Extracted with Dubos's solution, $CaCO_3$ added | tr. | + | + |

o, indicates no growth; tr., a trace; +, good growth.

The results were entirely negative when the *Sporocytophaga* was used, no signs of attack on the filter-paper being observed in any of the flasks even after 3 weeks.

This is in agreement with the established fact that *Sporocytophaga* is much more sensitive than *Cellulomonas* to the antibiotics in sawdust, even after neutralization of the acidic substances. Possibly a more complete extraction of the sawdust would have enabled the *Sporocytophaga* to develop and attack the filter-paper.

## Discussion

It is clear that antibiotics are present in sawdust, and it would seem that the inhibitory action towards the cellulose-decomposing bacteria is of two kinds. There is apparently an inhibition due to hydrogen ions, and also one due to a specific inhibitory substance or substances, but there is no certainty that the specific inhibitor is not merely the neutralized form of an acidic substance. However, there may well be a mixture of active substances in an extract of sawdust, and they may have a synergistic action. In view of the results obtained it is very possible that the specific antibiotics in sawdust are of the type which is more active under acid than under alkaline conditions, and they may well belong to the class of phenolic compounds which behave in this way (Lundy, 1938) and are known to occur in plant tissues (Onslow, 1929; Armstrong & Armstrong, 1931; Frykholm, 1945).

*Sporocytophaga myxococcoides* proved much more sensitive to the sawdust antibiotics than did the *Cellulomonas* species tested, and this is not surprising in view of the great sensitivity of the former organism to toxic substances. This organism was for long considered to be inhibited by low concentrations of substances such as glucose which are not usually regarded as possessing any toxic properties (Hutchinson & Clayton, 1919), but although this has now been shown to be erroneous, the toxicity being due to substances formed during the sterilization of the medium by heat (Stanier, 1942), it is evident that *Sporocytophaga* is inhibited by small amounts of substances which have little or no effect on the majority of bacteria. It was found that the activity of the sawdust antibiotics towards the *Cellulomonas* was reduced after autoclaving, whilst that towards the *Sporocytophaga* was apparently unaffected, but it may have been that substances toxic for the latter but not for the former organism were produced during the heat treatment of the sawdust extract, and that these offset a destruction of the original activity towards the *Sporocytophaga*.

It seems unlikely that the *Sporocytophaga* will ever prove of practical use for the decomposition of sawdust, since the organism proved unable to develop even when much of the inhibitory material had been extracted. The case is different with the *Cellulomonas*, since the addition of chalk, which is cheap and plentiful, would apparently suffice to reduce the antibacterial activity to a level low enough to permit development to occur. Acidity seemed to be the main reason why *Cellulomonas* failed to survive in untreated sawdust, and this aspect seems to have received little attention. Failure to recognize the acid nature of sawdust may have been

responsible for the conflicting reports as to its value for increasing the humus content of soils. It has long been known that the addition of lime to a compost heap aids its decomposition, but in this case only part of the cellulosic materials in plant residues are being decomposed by bacteria. No evidence has yet been obtained to show whether *Cellulomonas* species would be able to attack the cellulose in sawdust under otherwise favourable conditions, and even if it did the rate of attack might be too slow to be of use. In this connexion it should be noted that Virtanen (1946) has recently referred to the successful fermentation of cellulose in wood dusts by thermophilic and mesophilic bacteria. The fermentations were carried out in the presence of calcium carbonate, and the finer the wood was ground the greater was the decomposition. Fungi, with their greater powers of decomposition might be of more value, and an investigation of the antifungal properties of sawdust is now in progress. Some activity has been found, and here it may be recalled that Melin & Wikén (1946) have shown the presence of anti-fungal substances in pure forest litter from Swedish trees. Cold-water extracts of certain of these litters also contained heat-stable substances active against *Staph. aureus*, together with heat-labile substances capable of antagonizing their action. As sawdust extract did not become more active after heating, antagonistic substances were probably not present.

In conclusion it may be mentioned that it was observed on several occasions that in cultures of both *Cellulomonas* and *Sporocytophaga* in media containing sawdust extract the decomposition of the filter-paper appeared to be more extensive than in control cultures, even though there may have been an initial delay in the attack. Until quantitative tests are applied it cannot be considered to be a firmly established fact that sawdust extract contains stimulatory as well as inhibitory substances, but neither *Cellulomonas* nor *Sporocytophaga* is restricted to cellulose as a source of carbon (although the metabolic range of the latter is very limited) and the extract may well have contained available organic matter which aided multiplication and hence led to a later, more vigorous, decomposition of the filter-paper. Growth factors also may have been present and have assisted in the establishment of an active culture.

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(*Received 2 November* 1946)

# THE INFLUENCE OF LIGHT INTENSITY ON THE SUSCEPTIBILITY OF PLANTS TO CERTAIN VIRUSES

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(With Plate 6)

Reducing the light intensity under which plants were grown in summer to one-third increased their susceptibility to infection with tobacco necrosis, tomato bushy stunt, tobacco mosaic and tomato aucuba mosaic viruses. With the first two viruses shading increased the average number of local lesions per leaf by more than ten times and by more than five times with the second two.

Reducing the light intensity increased the virus content of sap from leaves inoculated with Rothamsted tobacco necrosis virus by as much as twenty times. As it also reduced the total solid content of sap by about one-half, purification was greatly facilitated; crystalline preparations of the virus were readily made from shaded plants but not from unshaded controls.

Reducing the light intensity also increased the virus content of systemically infected leaves; the greatest effect was with tomato bushy stunt virus with which increases of up to ten times were obtained, but with tobacco mosaic and aucuba mosaic viruses there were also significant increases.

The importance of controlled illumination in raising plants for virus work and the possible mechanisms responsible for the variations in susceptibility are discussed.

The symptoms produced by certain viruses depend greatly on the environmental conditions in which the host plants are grown. With some infections, variations in temperature and illumination are sufficiently important to determine whether infected plants behave as carriers or react severely. Except for their effects on symptom expression, however, environmental factors have received little attention in work on virus diseases, despite the fact that there are several diseases that show seasonal differences in their prevalence and in the ease with which they can be transmitted.

This seasonal variation is especially pronounced with tobacco necrosis. When Smith & Bald (1935) first described the disease, they commented on the fact that it is most prevalent in the Cambridge glasshouses during winter and early spring. Similarly, at Rothamsted it is common at that time of the year, whereas it is rare to see a naturally infected seedling during summer. In working on the isolation of the viruses causing tobacco necrosis, Bawden & Pirie (1942, 1945*a*) have also noted seasonal variations in the response of artificially infected plants. From November to February, plants usually react so severely that inoculated leaves become completely necrotic without the use of an abrasive to facilitate transmission. By contrast, during spring and autumn large numbers of lesions are obtained only when

an abrasive is used, and in summer relatively few lesions are produced even with the aid of an abrasive. The yields of virus from infected plants, and the ease with which it can be purified, also vary with the season. For example, using the tobacco necrosis virus they called the Rothamsted culture, Bawden & Pirie (1945*a*) have found that a litre of sap from the winter plants may contain between 100 and 200 mg. of virus and that this is readily prepared in a crystalline condition. In the autumn and spring, yields fall to about one-tenth of this and purification is much more difficult, while between May and September satisfactory preparations have not been made.

The type of lesion does not vary greatly through the year, and it seemed that these differences were reflexions of variation in the susceptibility of the host plants produced because of the different conditions in which they were growing. Of the various factors that might be concerned, it seemed probable that light intensity would be the most important. The experiments described in this paper show that reducing the light intensity under which plants are raised during the summer greatly increases their susceptibility to infection by several different viruses and also increases the concentration of virus attained in infected plants.

## Materials and methods

The viruses and hosts used were the Rothamsted culture of tobacco necrosis virus in tobacco, var. White Burley; tomato bushy stunt virus in *Nicotiana glutinosa* and tomato, var. Kondine Red; tobacco mosaic virus in *N. glutinosa* and tobacco; and tomato aucuba mosaic virus in tobacco and tomato.

No attempt was made to grow plants under constant or standard light conditions. The experiments were made between the beginning of May and the end of September, and one half of the plants were kept in the ordinary fluctuating glasshouse conditions while the others were kept under reduced light conditions by shading with a covering of closely woven white muslin. This was done by enclosing the plants in light wooden frames, 24 in. high and with sides 34 in. long, covered with a loosely fitting hood of the muslin. The seedlings used were all raised under the same conditions and were transferred to the differential light conditions at the time they were transplanted from the seed-pans to 8 in. pots. Sixteen pots were contained in each cage and the same number of controls were kept near to the cage in the same glasshouse. The light intensity was measured by a lumeter on various occasions when in the glasshouse it varied between 200 and 20,000 f.c. At each occasion the light intensity in the cages was approximately one-third of that outside. The air temperature in the cages did not vary from that outside by more than 1° C. The control plants were only rarely exposed to direct sunlight, for the weather throughout the period of the experiments was unusually dull; also the slat blinds of the house were pulled down whenever normal glasshouse practice required it. About 10 days after transplanting, each plant was given a top dressing of liquid

fertilizer containing 0·1, 0·06 and 0·1 g. of nitrogen, phosphorus pentoxide and potassium oxide respectively. Plants were kept for about 4 weeks in the differential light conditions before being inoculated.

Inoculations were made by rubbing whole leaf surfaces with the forefinger wet with inoculum. When kieselguhr was used as an abrasive to facilitate transmission, it was lightly dusted over the leaves before they were rubbed. Serological tests were made by methods previously described (Bawden & Pirie, 1942).

### Effect of shade on growth and constitution of host plants

The light intensity in the cages was sufficient throughout the period to prevent the plants from becoming seriously etiolated. As would be expected, however, they differed considerably from the control plants both in appearance and in constitution. In general the plants in the cages were taller, had larger but thinner leaves, were more succulent, and a deeper green than the controls, which often showed signs of nitrogen deficiency on the lower leaves while the shaded plants were uniformly green. Several sets of equivalent leaves from the caged and control plants were compared for various properties and one such comparison made with tobacco plants in June is shown in Table 1. In this eighteen leaves were picked from each

Table 1. *Effect of shade on the properties of uninfected tobacco leaves in June*

| Property | Shaded leaves | Unshaded leaves |
|---|---|---|
| Average leaf area (sq.cm.) | 198 | 136 |
| Average leaf weight (g.) | 5·3 | 4·8 |
| Dry weight as % wet weight | 9·3 | 15·9 |
| Nitrogen as % wet weight | 0·73 | 0·63 |
| Nitrogen as % dry weight | 9·85 | 5·04 |
| Volume of sap from 100 g. leaf (ml.) | 80 | 70 |
| Volume of sediment from 100 ml. sap (ml.) | 30 | 36 |
| Dry weight per ml. of clarified sap (mg.) | 30 | 52 |
| Nitrogen per ml. of clarified sap (mg.) | 1·29 | 1·36 |

set of plants, weighed, minced in a meat mincer, frozen, thawed, squeezed through muslin and the sap clarified by centrifugation. It will be seen that the leaves from the shaded plants had a larger wet weight but smaller dry weight than leaves from unshaded plants; they gave a greater volume of sap, which contained about half as much dry matter as sap from the unshaded plants, but about the same amount of nitrogen. Clarified sap forms the starting point for most work on the isolation of viruses and the solid content of this was reduced from 52 to 30 mg./ml. by shading. The increase in leaf area of the shaded plants shown in Table 1 was about usual for tobacco in the first experiments, but later and with *N. glutinosa* and tomato the increase was smaller. Bawden & Pirie (1945*a*) have shown that much virus remains attached to the fibrous residues of the leaf remaining after sap has been expressed, and that some of this can be released by grinding the fibre finely in a triple roller mill. Less fibre is obtained from shaded leaves than from unshaded leaves and

when equal weights of fibre from both kinds are milled and extracted with equal volumes of water, the solid content of mill extracts from the shaded is usually about two-thirds of that of extracts from unshaded leaves. Most of the extra material obtained from the unshaded leaves is removed by freezing, thawing and centrifuging, and after such treatments the mill extracts of both kinds of leaves contain similar amounts of solids.

### Tobacco necrosis virus

Table 2 shows the results of comparing the relative susceptibilities of shaded and unshaded tobacco leaves to infection with the Rothamsted culture of tobacco necrosis virus. Clarified sap from infected tobacco leaves diluted in water was used as inoculum and at least fifteen leaves were inoculated for each comparison. The results show that shading increased the number of lesions produced by from four to nineteen times, with an average increase of 13·5 times, a factor too great for the

Table 2. *Effect of shade in increasing the susceptibility of tobacco leaves to tobacco necrosis virus*

| Time | Inoculum | Average no. of local lesions per half leaf on: Shaded leaves | Unshaded leaves | Ratio shaded/unshaded |
|---|---|---|---|---|
| June | 1/20, no abrasive | 88 | 22 | 4 |
| | 1/200, no abrasive | 46 | 5 | 9 |
| | 1/200, with abrasive | 105 | 8 | 13 |
| | 1/2000, with abrasive | 23 | 1·5 | 15 |
| July | 1/20, no abrasive | 22 | 1·3 | 17 |
| | 1/200, no abrasive | 7·5 | 0·4 | 19 |
| | 1/200, with abrasive | 64 | 4 | 16 |
| | 1/2000, with abrasive | 14 | 1 | 14 |

increased area of the shaded leaves to be worth considering. Unshaded leaves are much tougher than shaded ones, and it was thought that part of their greater resistance might lie in the fact that the thick epidermis was insufficiently affected by rubbing to produce entry points. Comparative tests were made, therefore, with kieselguhr as an abrasive to determine its effect with both kinds of plant. It will be seen that it greatly increased the numbers of lesions obtained on both kinds of plants, but did not reduce the difference in susceptibility between the two types of leaves.

Only necrotic local lesions are obtained when tobacco leaves are infected with tobacco necrosis viruses, so that the yield of virus obtained from inoculated plants will depend on the number of local lesions produced. Hence the fact that more lesions are produced with the same inoculum on shaded than on unshaded plants could alone go far towards explaining the differences in yield of these viruses obtained in summer and winter. The figures given in Table 2, however, were

obtained with relatively dilute inocula, deliberately chosen to give results in the range where numbers of lesions are related to virus concentration, and the results might not apply directly to the conditions usually employed in infecting plants for the production of virus. Comparisons were therefore made in which equal numbers of equivalent leaves on shaded and control plants were dusted with kieselguhr and rubbed with undiluted infective sap. Three such tests were made, in June, July and the end of September respectively. In all three, the shaded plants produced many more lesions than the unshaded, but there was considerable variation in the results from experiment to experiment. In June, although there were large numbers of lesions on the shaded leaves, these were insufficient to coalesce, while on the controls there were only a few scattered lesions. In the other two tests, the lesions coalesced on the shaded plants, whereas in the controls they did not though there were more lesions in July than in June and many more in September.

The control plants in June gave too few lesions for any comparative attempt at isolation of the virus to be worth while. Clarified sap from the shaded leaves when tested serologically gave a precipitin titre of 1 in 12, indicating a virus content of about 30 mg./l., whereas undiluted sap from the unshaded plants contained too little virus to give a visible precipitate with antiserum. From the plants inoculated in July equal numbers of leaves were taken from shaded and unshaded plants; after cutting out the coarsest part of the mid-ribs, the laminae were minced, and the sap was expressed through muslin and frozen. After thawing and centrifuging at 3000 r.p.m., the supernatant fluids from the shaded and unshaded leaves contained respectively 62 and 38 mg. of dry matter per ml. Equal volumes of clarified sap from the two lots of leaves were taken for purification of the virus. After precipitating the virus three times with $\frac{1}{4}$-saturated ammonium sulphate solution, the preparations were centrifuged at *p*H 4·5. The supernatant fluid was then adjusted to *p*H 7 and the virus in it precipitated with ammonium sulphate. The precipitate was dissolved in water, dialysed, and insoluble material removed by low-speed centrifugation, when the solution was ultra-centrifuged for 1 hr. at 40,000 r.p.m. The pellets obtained from the preparation made from the shaded leaves were colourless and when examined microscopically seemed to be wholly crystalline (Bawden & Pirie, 1945*a*), whereas those from the unshaded leaves were smaller, brown and showed only a trace of crystallinity at their fringes. The yields of final product from the two leaves were equivalent to 60 mg./l. of clarified sap from the shaded plants and 7 mg./l. from the unshaded. As the precipitin titre of the preparation from the unshaded leaves was only 1/150,000 compared with 1/300,000 for that of the preparation from shaded leaves, it is probable that there was twenty times as much virus in the first as in the second.

The two lots of plants used in the experiment at the end of September (Pl. 6, fig. 1) showed smaller differences in appearance than those used earlier and this was reflected in the larger number of lesions obtained on the unshaded controls. Sap from the two lots of plants was treated in the same manner as in the previous

experiment, and again a wholly crystalline pellet was obtained from the shaded plants whereas that from the unshaded plants was only partly crystalline. The final yields from the shaded and unshaded plants were equivalent respectively to 100 mg./l. and 20 mg./l. of clarified sap.

Whether or not there are other factors concerned in affecting the seasonal variations of tobacco necrosis, it is clear that light intensity alone is sufficient to explain most of the differences. By reducing illumination during the summer to one-third of the normal, the same inoculum produces more than ten times as many lesions, shaded plants give more sap, which may contain twenty times as much virus and only half as many other constituents as sap from unshaded plants. The light intensity under which the shaded plants were grown in these experiments was greater than that experienced by winter plants in the glasshouses at Rothamsted, when illumination is the limiting factor influencing growth. The shaded plants did not develop any natural infections nor did the inoculated leaves collapse with the coalescing of local lesions, as is common with winter plants. Also, the yields of purified virus were below those frequently obtained in December and January. Hence it seems that susceptibility to infection might have been increased still further had the light intensity been further reduced.

### Tomato bushy stunt virus

Tomato bushy stunt resembles tobacco necrosis virus in showing a seasonal variation in intensity and in affecting young plants much more severely than old ones. Young tomato plants infected in the winter rapidly develop a severe mosaic with necrotic spotting that may be fatal. In summer, and with older plants, local lesions only are sometimes obtained, and systemic symptoms are less severe, consisting usually of scattered chlorotic and necrotic spots. In *N. glutinosa* necrotic local lesions are the only symptom, but many more are produced on young than on old leaves, and winter plants give more than summer ones.

The effect of light intensity on the susceptibility of *N. glutinosa* to tomato bushy stunt virus was tested on two occasions, in June and August. On each occasion purified virus was used as inoculum at two dilutions, $10^{-5}$ and $10^{-6}$ mg./ml., and at least 40 equivalent half-leaves were inoculated for each comparison. The results are summarized in Table 3, which shows that shading increased the number of lesions on average by about twelve times (Pl. 6, fig. 2). In both the shaded and unshaded plants, there was a gradient of increasing susceptibility from the oldest to the youngest leaves, but at all levels the shaded plants gave more lesions. The type of lesions also varied with the age of the inoculated leaf. The largest lesions occurred on the young leaves and consisted of a wholly necrotic spot with a brownish centre surrounded by a black ring. On the middle leaves the lesions were smaller and consisted of a pin-point necrosis with a chlorotic halo, while on the bottom leaves the few lesions obtained were almost wholly chlorotic. When lesions of the different types were isolated and used as inoculum for infecting other *N. glutinosa*,

each type gave rise to all three types merely depending on the age of the leaf inoculated.

Table 3. *Effect of shade on the susceptibility of* N. glutinosa *to tomato bushy stunt virus*

| Time | Dilution of virus | Average no. of lesions per half leaf on: Shaded leaves | Unshaded leaves | Ratio shaded/unshaded |
|---|---|---|---|---|
| June | $10^{-5}$ | 25·5 | 3·9 | 6·5 |
| | $10^{-6}$ | 8·7 | 0·7 | 12·4 |
| August | $10^{-5}$ | 23 | 1·5 | 15·3 |
| | $10^{-6}$ | 4·5 | 0·3 | 15 |

The effect of light intensity on the multiplication of tomato bushy stunt virus was tested on two occasions in tomato plants. Tomato plants grown under the cages were more obviously affected by shading than were tobacco plants and they showed definite signs of etiolation. They were half as tall again as the control plants, had smaller and paler leaves, and were much weaker. Equal numbers of caged and uncaged plants were inoculated in June with undiluted infective sap; all the leaflets on the three lowest leaves of each plant were rubbed after dusting with kieselguhr. Both lots of plants produced local lesions, but these were more abundant and more severe on the shaded plants, which wilted and died within a fortnight. Three weeks after inoculation, when systemic symptoms were apparent though not severe, the young leaves from both lots of plants were picked, minced and the sap expressed and frozen. The sap was then fractionated as already described for tobacco necrosis virus, except that it was not ultra-centrifuged, and the virus content of the final preparations estimated by serological tests. The shaded plants gave four times as much virus as the unshaded, and the results indicated the virus content of the original saps to be respectively about 80 and 20 mg. of virus per litre.

A similar test was made in August and this time sap from the shaded plants contained about 150 mg./l., more than ten times the amount in sap from control plants. In this test a comparison was also made of the amounts of virus liberated from the fibrous leaf residues by milling. 10 g. of fibre remaining after the expression of sap from both lots of plants were passed twice through the triple-roller mill and then extracted with 20 ml. of water. The extracts were frozen, thawed and centrifuged, and then tested serologically, when the extract from the shaded plants gave a precipitin titre eight times as great as the extract from the unshaded plants.

### Tobacco mosaic and aucuba mosaic viruses

In working with tobacco mosaic virus or with the related strain that causes aucuba mosaic of tomato, no difficulties are experienced in getting infections on susceptible plants throughout the year. Nevertheless, our results show that light intensity does influence susceptibility to infection and the concentration of virus attained in

infected plants, though less so than with tobacco necrosis and tomato bushy stunt virus. Table 4 shows this increase in susceptibility as found in local lesion tests with tobacco mosaic virus in *N. glutinosa* and aucuba mosaic virus in tobacco. Purified virus preparations were used as inocula and at least thirty half-leaves were inoculated for each individual comparison. It will be seen that in all comparisons shading increased the numbers of local lesions, though there were large variations in the amount of increase in different tests.

TABLE 4. *Effect of shade on susceptibility of* N. glutinosa *to tobacco mosaic virus and of tobacco to aucuba mosaic virus*

| Time | Inoculum | Average no. of lesions per half leaf on: Shaded leaves | Unshaded leaves | Ratio shaded/unshaded |
|---|---|---|---|---|
| May | *T.M.V., $10^{-5}$, no abrasive | 67·7 | 31·6 | 2·1 |
| | T.M.V., $10^{-6}$, no abrasive | 23·4 | 5·9 | 4 |
| | T.M.V., $10^{-7}$, abrasive | 15·6 | 1·5 | 10 |
| July | T.M.V., $10^{-5}$, no abrasive | 43·7 | 5·5 | 8 |
| | T.M.V., $10^{-6}$, no abrasive | 14·6 | 1·5 | 10 |
| June | A.M.V., $10^{-5}$, no abrasive | 56 | 41 | 1·4 |
| | A.M.V., $10^{-6}$, no abrasive | 28 | 18 | 1·6 |
| | A.M.V., $10^{-6}$, abrasive | 165 | 90 | 1·8 |
| | A.M.V., $10^{-7}$, abrasive | 46 | 31 | 1·5 |
| August | A.M.V., $10^{-5}$, no abrasive | 90 | 10 | 9 |
| | A.M.V., $10^{-6}$, abrasive | 285 | 40 | 7 |

* T.M.V. tobacco mosaic virus; A.M.V. tomato aucuba mosaic virus. Dilutions given as g. of purified virus per ml.

The effect of light intensity on the local multiplication of tobacco mosaic virus was tested late in July, when equivalent leaves of shaded and control tobacco plants were dusted with kieselguhr and rubbed with a purified virus solution containing 1 mg./ml. Twelve days later the inoculated leaves were picked, minced, and the sap expressed. The sap was heated to 60° C. to aggregate the virus particles (Bawden & Pirie, 1945*b*) and to coagulate most of the normal plant constituents. After centrifuging, the two clarified saps were compared for the extent to which they showed anisotropy of flow when shaken between crossed 'polaroids' and for their precipitin titres. The sap from the shaded plants showed anisotropy the more strongly and gave a precipitin titre of 1/1000 compared with 1/500 for that of the control.

The increase in virus content of inoculated leaves produced by shading could merely reflect the fact shown in Table 4 that tobacco mosaic virus establishes itself at more separate points in shaded than in unshaded leaves. In other words, the virus content of inoculated leaves may be a direct function of the number of local lesions. Tests were therefore made to see whether illumination affects the virus content of systemically infected leaves. Equal numbers of shaded and control

tobacco plants were inoculated on two lower leaves in July and the young leaves were picked 4 weeks later when they were showing good symptoms of systemic infection. The mid-ribs were discarded and the laminae minced, the sap expressed and frozen overnight. After thawing and centrifuging, the supernatant fluid from the shaded plants showed stronger anisotropy of flow than the control; after heating samples to 60° C. and centrifuging, both lots of sap showed increased anisotropy of flow and when tested serologically that from shaded plants gave a precipitin titre of 1/3000 and the control one of 1/1500. This difference was confirmed by the yields of purified virus obtained from frozen sap. After isolation by the method described by Bawden & Pirie (1943), yields of virus from clarified sap were 4·5 and 3·2 g./l. respectively from the shaded and unshaded plants. As the total solid content of the sap from shaded and control plants after freezing and centrifuging was 40 and 53 g./l., the ratio of virus to other material in the clarified sap from shaded leaves was nearly double that of the control. The fibrous residues from the shaded plants also seemed to contain more virus than those of the control plants, though no attempt was made to find the total virus content of the plants. Samples of 25 g. of leaf residue from the two types of plant were milled, extracted with 50 ml. of water and the virus content of these extracts determined. The extracts were frozen overnight, thawed and centrifuged and the virus isolated from the supernatant fluids, when 2·5 g./l. were obtained from the mill extract of the shaded leaves and 1·8 g./l. from the unshaded.

Similar results were also obtained from tests with aucuba mosaic virus in tomato. The first, made in June, showed that the sap from systemically infected leaves of shaded plants, after clarifying by heating to 60° C. and centrifuging, showed stronger anisotropy of flow than similarly treated sap from controls. The sap from shaded and unshaded plants gave precipitin titres of 1/2500 and 1/1500 respectively, and when the virus was isolated from the two yields were obtained equivalent to 1·53 and 1·19 g./l. respectively. In a second test made in August, in which the fibrous residues of the tomato plants were milled and extracted, both the sap and the mill extract from the shaded plants contained more virus than similar fractions from the control. The virus content was not measured by isolation and weighing, but after clarifying by heating and centrifuging the sap and mill extracts from the shaded plants showed the greater anisotropy of flow and both gave precipitin titres of 1/1500 compared with 1/1000 and 1/800 for the sap and mill extracts from the unshaded plants.

## Discussion

From the results described in this paper it seems that the conditions under which plants to be used in virus studies are raised merit more attention than they have received in the past. Variations in these conditions at different times of the year or from place to place could account for many of the apparent contradictions found in the literature concerning such properties of viruses as dilution end point, ease of transmission and susceptibility and resistance of different plants to infection. The

importance of the environment will almost certainly vary greatly with different viruses, but susceptibility to all four used in this work was increased by reducing the intensity of illumination below that at which plants are normally grown. Along with increased susceptibility to infection, a higher virus content was attained in the infected plants, which at the same time contained less other materials than plants receiving more intense illumination. Hence for the production of purified virus preparations, shading the plants is very advantageous: it increases ease of infection, yield of virus and ease of purification. In raising plants for local lesion tests the main criterion is uniformity, but in sets of plants raised under apparently identical conditions occasional leaves upset the uniformity by producing several times more lesions than others when rubbed with the same inoculum. Though there may be other factors involved, it seems likely that these anomalous leaves may be ones that have been shaded by others and so have had their susceptibility increased.

There is no positive evidence to indicate the mechanism whereby alterations in light intensity affect susceptibility to viruses. Some of the effects may be simply mechanical. Shaded leaves are much more fragile than those exposed to bright light, so that they are probably more readily injured during inoculation and provide a correspondingly greater number of entry points for the virus. However, there is probably much more to the story than this. If the effect were simply a mechanical one determining the number of wounds made in the epidermis, it might explain the increase in number of local lesions and the increase in virus content of inoculated leaves, but it could not account for the differences found in systemically infected leaves. Such leaves become infected by spread of viruses inside the plant and without any need for abrasions of the epidermis, yet systemically infected leaves from shaded plants consistently yield sap with a higher virus content than that from control plants.

The most likely explanation of the difference would seem to be that some product or products of photosynthesis interfere with virus multiplication, which is thus able to proceed more smoothly in shaded plants. It is worth noting that shading produces a greater effect with tobacco necrosis and tomato bushy stunt viruses than with tobacco mosaic and aucuba mosaic viruses. The susceptibility of plants to the first two also shows much greater change with age than to the second two, and on the same plants there is also a striking gradient of increasing susceptibility from the oldest to the youngest leaves. One of the most noticeable effects of shading is to delay the onset of symptoms of maturity, which show in the controls in coarser textured, thicker, and more chlorotic leaves. Obviously the older the plant, and the older the individual leaves on one plant, the more chances they will have to accumulate any products of photosynthesis that may interfere with virus multiplication. We know so little of the mechanism of virus multiplication that any attempt to explain the phenomenon further can be only highly speculative. However, it is known that viruses readily combine with many different kinds of substances and that some of these are highly efficient inhibitors of in-

fectivity. It is also possible that the first stage in the initiation of infection in a cell entails the combination of virus with some specific cell component. Thus in conditions where photosynthesis has proceeded at a high level or for a long time there may be an accumulation of substances which combine readily with the viruses and prevent them from becoming attached to their normal sites of multiplication. The increased susceptibility induced by reducing light intensity may be partly mechanical by increasing ease of entry into inoculated leaves, but may also be partly an intracellular phenomenon because of the reduced formation of cell contents that interfere with the ready establishment of virus particles in conditions necessary for multiplication. On this view some products of photosynthesis would act in a manner analogous to antibodies, so that the more of them a cell contained the more virus would need to be introduced before their neutralizing effects were overcome and active multiplication could begin.

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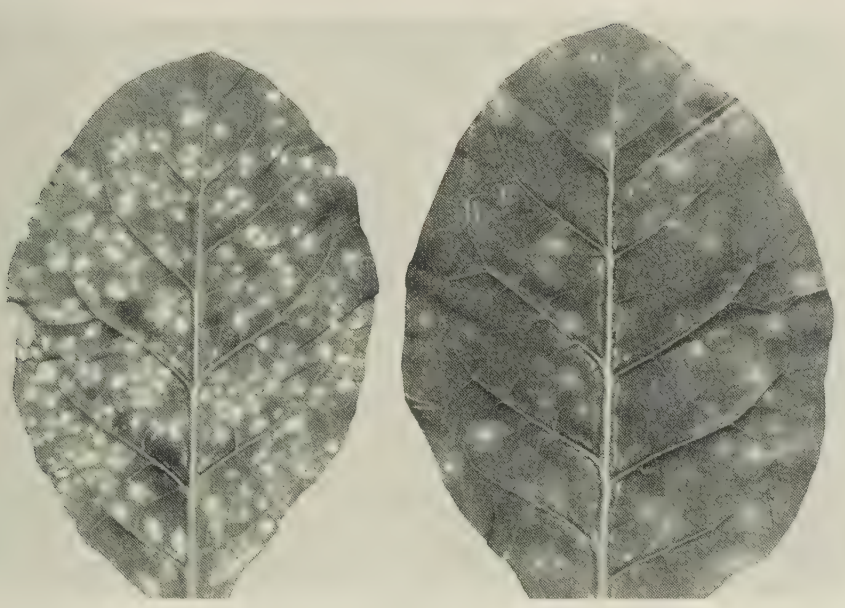
## EXPLANATION OF PLATE 6

Fig. 1. Tobacco leaves inoculated in September with Rothamsted tobacco necrosis virus. (*a*) shaded leaf, (*b*) unshaded leaf.

Fig. 2. Leaves of *Nicotiana glutinosa* (*a*) from shaded plant, (*b*) from unshaded plant inoculated with tomato bushy stunt virus in July. Left hand half-leaves inoculated with virus at $10^{-5}$ g./l. and right hand half-leaves with virus at $10^{-6}$ g./l.



*a* *b*

Fig. 1

*a* *b*

Fig. 2

BAWDEN and ROBERTS—*Influence of light intensity on the susceptibility of plants to certain viruses*

# PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 8 November 1946, in the Imperial College of Science and Technology, London; the President, Dr C. B. Williams, in the Chair.

The following paper was read and discussed:

Rodent control in towns. By S. A. Barnett.

Ordinary Meeting of the Association held on Friday, 6 December 1946, in the Imperial College of Science and Technology, London; the President, Dr C. B. Williams, in the Chair.

The following papers were read and discussed:

1. Potato haulm destruction with special reference to the use of tar acid compounds. By A. R. Wilson, A. E. W. Boyd, J. G. Mitchell and W. S. Greaves.

2. Recent developments in chemical methods of weed control. By Prof. G. E. Blackman.

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## RODENT CONTROL IN TOWNS

By S. A. Barnett, *Ministry of Food Infestation Control*

(With 2 Text-figures)

### 1. Introduction

The problem of rodents in stored food is probably as old as civilization, since civilization, with the growth of towns, depends on the existence of an agricultural surplus; and this surplus has to be stored. Throughout history the main method of dealing with rodent pests has been a form of biological control, mainly by dogs and cats but sometimes by other predators. Other methods, such as haphazard poisoning and trapping, are also of long standing.

The limited success of traditional methods has doubtless helped in the growth of the many fables supposed to illustrate the cunning and ingenuity of rats. Rats have been said to warn other rats of the danger of poison bait, to avoid traps that have been handled by man, and to collect their food in a variety of ingenious ways, some involving co-operation between individuals. An extensive mythology has flourished in the absence of any thoroughgoing scientific study of wild rat behaviour.

It was not until September 1939, under the pressure of war needs, that a systematic investigation was begun. Through the Agricultural Research Council it was arranged that the Bureau of Animal Population at Oxford, directed by Charles Elton, should devote its energies to research on the problems of rodent control. The results achieved were put into practice by the various interested Ministries and by the local authorities.

As far as urban areas are concerned, only three species are involved: *Rattus norvegicus* Berkenhout, the common brown rat; *R. rattus* L., the so-called black or ship rat; and *Mus*

*musculus* L., the house mouse. The present paper describes certain aspects of the wartime work of the Ministry of Food Infestation Division against these species. The Ministry was responsible not only for dealing with infestations in food stocks under its own control, but also for administering rat destruction in all types of premises on non-agricultural land. On the scientific side the Infestation Division occupied an intermediate position between the research workers at Oxford and those carrying out the control work itself (see Gracie, 1944).

## 2. Methods

The research on which the Ministry's methods are based is described in the *Reports*, now in preparation, of the Bureau of Animal Population. A summary of the methods has been published in the form of a handbook (Ministry of Food, 1946). A full account of the methods and results of the Ministry is in preparation.

Of the possible methods, including poisoning, trapping, fumigation and the use of predators, poison baiting is the one mainly used. Successful poison baiting requires the application of four principles: (1) plain bait must be put down for several days before poison bait is laid ('prebaiting'; see Doty (1945) as well as *Bureau of Animal Population Reports*); (2) in successive treatments both the non-poisonous part of the bait (the bait base) and the poison itself must be changed; (3) only bait bases and poisons of known efficacy should be used; (4) the whole area of an infestation must be treated at once, irrespective of the ownership or tenancies of land or buildings ('block control').

The first of these principles arises from the tendency of rats to avoid unfamiliar objects, including new food. This aversion is gradually overcome, sometimes over a period of several days. It may be caused by any new thing, dangerous or not, in the environment. It is, of course, responsible for many of the stories of the 'intelligence' of rats. When poison bait is laid without preliminary baiting with plain bait, this avoidance may lead to the ingestion of only sublethal doses by many of the rats, and consequent failure of the baiting.

The second principle also arises from the possible existence of rats which have taken sublethal doses. Rats, like men, tend to reject a food which has caused a physiological disturbance, if it is offered again within a few weeks. Even after a well-planned campaign there may be such bait-shy rats present, and these must be poisoned with different materials.

Table 1. *Relative toxicities of materials used in rodent control*

| Poison | Approx. LD 50 mg./kg. |
|---|---|
| Zinc phosphide $Zn_3P_2$ | 40 |
| Arsenious oxide $As_2O_3$ | 190 |
| Antu (alphanaphthylthiourea) | 4·5 |
| Red squill powder | 450 |
| Barium carbonate $BaCO_3$ | 750 |

The figures for toxicities are based on experiments done on an inbred strain of albino rats kept at Oxford; they are expressed in mg./kg. body weight (see *Bureau of Animal Population Reports*).

The third principle has only become applicable during the recent war. It is now possible to make a fairly accurate estimate of the percentage kill that can be expected from a given control measure (Chitty, 1942; Chitty & Shorten, 1946). By using this method it has been possible to show that certain bases and poisons are highly effective against *Rattus norvegicus* in this country. Table 1 lists the poisons now recommended; it also includes barium carbonate, which was extensively used until it was shown to be unreliable. The bait bases used are sausage rusk* mixed with its own weight of water, national flour plus sugar (10% by weight), bread and water, and wheat soaked in water.

The fourth principle is perhaps the most obvious of all. It is of great administrative importance, and sometimes raises quite serious problems. The main technical problem arises

* A biscuit meal used as a filler in sausages.

from the fact that sewer systems are generally infested with *R. norvegicus*. It is often found to be the case that rats are moving freely from infested premises into the sewers and back again. This situation leads to the necessity for simultaneous baiting of sewers and surface premises. This procedure is called 'vertical block control'.

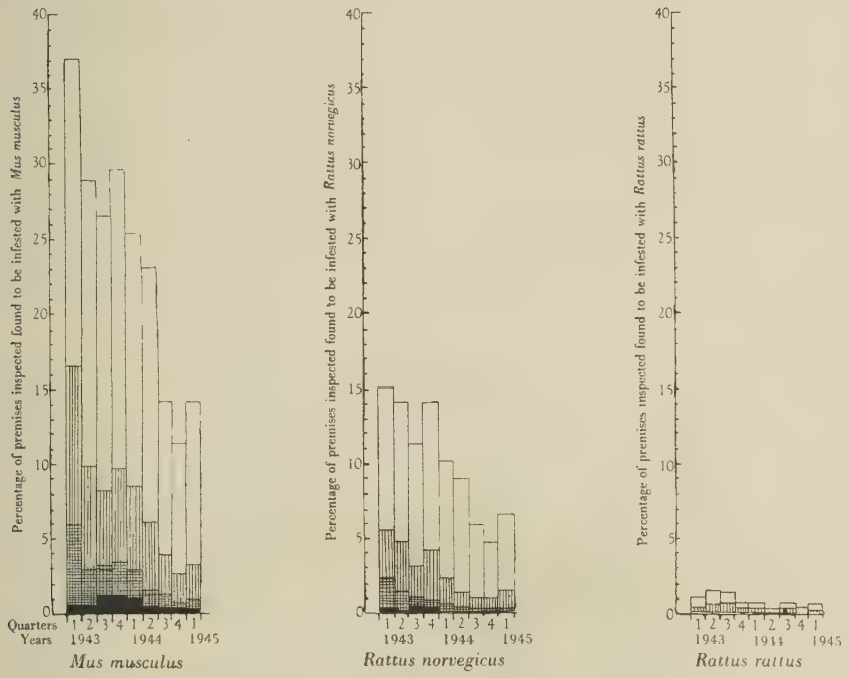

Percentage of premises inspected found to be infested with *Mus musculus*
Percentage of premises inspected found to be infested with *Rattus norvegicus*
Percentage of premises inspected found to be infested with *Rattus rattus*
0 5 10 15 20 25 30 35 40
Quarters 1 2 3 4 1 2 3 4 1
Years 1943 1944 1945
*Mus musculus*
*Rattus norvegicus*
*Rattus rattus*
Ministry of Food Buffer and Sub-Depots—Jan. 1943 to Mar. 1945. Reduction of infestation with rats and mice
Key to infestation: Very heavy. Heavy. Medium. Small.

Fig. 1

## 3. Control measures

### (*a*) *Ministry of Food stores*

During the period 1943–5, the stocks owned by the Ministry, apart from those in cold stores, were kept in about 7500 depots. Fig. 1 illustrates certain features of the rodent infestation of these stores from January 1943 to March 1945. During this period, not only was the number of food stores fairly constant, but the food stocks themselves were kept without very great variation in quantity or character. It is therefore legitimate to make comparisons of the degree of infestation in them at different dates. Later the situation changed so much, both in the amount and character of food in the country and in the length of time during which any given consignment was stored, that valid comparisons could no longer be made.

The period covers the first nine quarters during which the Ministry possessed an effective staff for inspection and control. For each store inspectors were required to give an indication of the degree of infestation found, as well as the species. Infestations were classified as *small*, *medium*, *heavy* or *very heavy*. No precise definitions for these four terms

were given, and doubtless there was individual variation in their use. Moreover, as infestations became less heavy and less numerous, there may have been an unconscious change of standard, so that, for example, infestations which would previously have been reported as 'medium' came to be reported as 'heavy'. It might therefore be thought better to ignore the degree of infestation and to record only the proportion of stores infested. But to do so would be misleading, since a 90% reduction in the rodent population of the stores could be accompanied by only, say, a 40% reduction in the proportion of stores found infested. While it is still impossible, in so large a mass of stored food, to prevent infestation entirely, the results of control show that infestations can for the most part be kept small.

Fig. 1 shows the reported infestation of Ministry stocks, together with the estimated density of infestation. The total figures are the percentages of inspected premises found infested. The percentage of all stores which were infested would be lower, since infested stores were more likely to receive a visit than uninfested. From the figure it will be seen that, of the three species, *R. rattus* could almost be ignored as a serious pest in these stores. This species is distributed mainly in the ports of Britain (Matheson, 1939), and the Ministry's stores were, on the whole, away from target areas such as ports and the major industrial centres. The other species of rat, *R. norvegicus*, was undoubtedly a serious menace in 1943, but by 1945 it had been brought well under control, and even 'medium' infestations were quite rare.

Mice have proved, for reasons to be discussed more fully elsewhere, the most difficult of the three species to deal with. It is possible that this is partly because the destruction of rats has released opportunities for nesting and feeding, but the main reason is the capacity of mice to develop large colonies entirely within stacks of sacked cereals. However, even this species was greatly reduced in numbers during the period under review.*

To draw valid and definite conclusions from these figures, a control would be required. However, fortunately for our food supply, there were no food stores in which rodents were allowed to multiply unchecked. We may therefore ask the reasonable question: what would have happened if no organized and scientific destruction had been carried out? But we can only guess at the answer.

### (*b*) *Other surface premises*

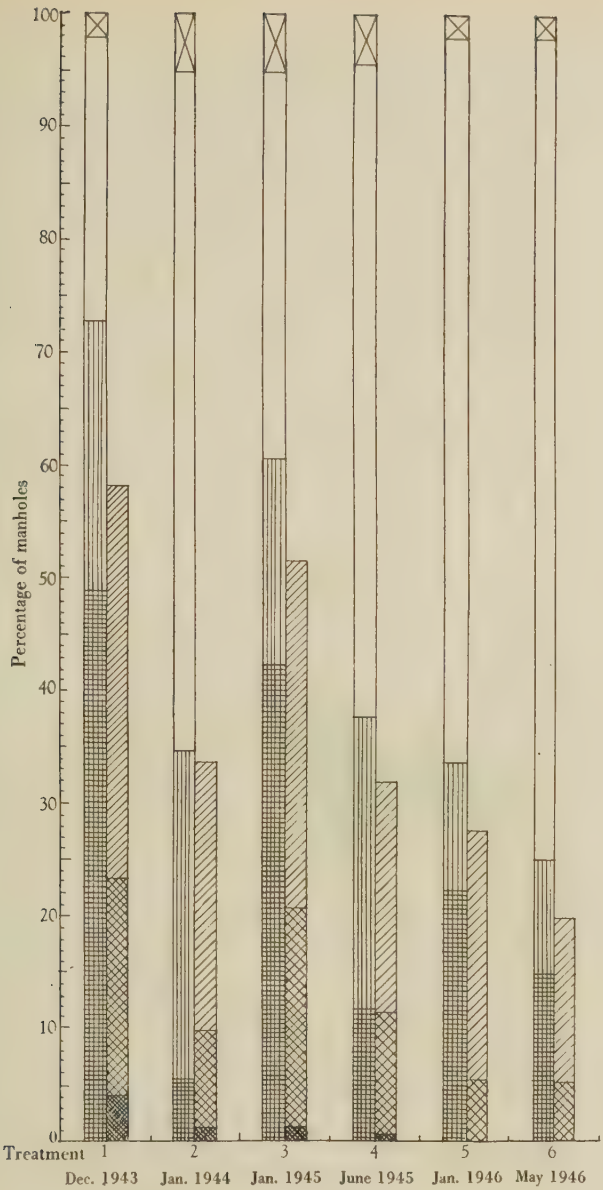
Apart from the premises controlled by the Ministry itself, special attention was given to those in which conditions inevitably encourage rat infestation. Most of these premises belong to the category of *offensive trades*: they include glue works, salvage destructors, and abattoirs. (Refuse tips are also an important class of vulnerable sites.) In 1945 there were rather more than 1700 premises on which offensive trades were carried out. It was arranged that all these premises should be inspected, and that, where infestation was found, steps should be taken to get rid of it.

To-day, 1252 of these premises are reported to be clear, or to have only minor infestations (that is, infestations estimated to consist of less than twenty rats). 341 were found to have infestations of a more minor character and most of them have been dealt with. 131 are still under investigation. When all these premises have been finally cleared, or at least, when their infestations have been brought under control, an important source of rat infestation will have disappeared.

### (*c*) *Sewers*

Even more important than these special trades, as a source of infestation in all types of surface premises, are the sewers. All old systems are infested with *R. norvegicus*: only very recently constructed systems, or those which are regularly flooded, sometimes escape infestation; small diameter pipes are no bar. The rats do not nest in the sewers themselves, but probably in the surrounding earth, in dry sealed off drains or side connexions, and

* Since March 1945 it has become the practice to fumigate, where possible, stores in which serious infestations of mice develop.




Urban Authority No. 1.
Sewer manhole baiting results

Key—'C' prebait takes
Total prebait takes
Not infested
Not baited
'C' poison takes
'C + G' poison takes
Total poison takes

Fig. 2

possibly in surface premises. Movement in and out of sewers occurs through defects in the walls of brick sewers, through broken drain pipes, or through defective house drains.

The destruction of sewer rats presents a special problem because, although we may make a guess at where they are nesting, it is never possible to get a clear picture of the movements of sewer rats in any particular instance. When surface premises are baited, the success of the treatment depends largely on the extent to which the rats are intercepted between their nesting sites and their source of food. No such accurate interception is possible in sewers. Baiting has to be done in the manholes.

Fortunately, a series of experiments showed that, with adequate organization, a high percentage kill could be expected in the sewers, as in surface premises, after prebaiting. The details of this work will appear elsewhere. Here it is only possible to give some illustrations of the scale of war-time sewer rat control.

The biggest single operation was in London. In the area of the London County Council (together with certain regions outside its boundaries) the whole sewer system is continuous on each side of the Thames: there are consequently two vast interconnected systems, together about 2700 miles long, each of which was, until 1944, heavily infested with rats. In the period December 1943 to February 1944 a combined onslaught, directed by the Ministry of Food, was made on these rats by the forty-four local authorities concerned. 1150 men were employed for 10 weeks. About 38,000 manholes were baited. Two successive treatments, involving prebaiting and poisoning with different materials in each, were given. In the first 56·7% of manholes baited showed take of poison bait; in the second, 37·8%. This figure indicates a reduction in the infested area of about one-third.

The reduction in the total rat population was, however, probably much greater than the reduction in the infested area. Evidence for this is obtained by considering heavily infested manholes, that is to say, those at which poison takes were recorded as 'complete' or 'more than half'. In the first treatment these two categories together accounted for 27·2% of manholes; in the second, 9·7%. Thus the reduction in the total rat population by the first treatment was clearly greater than the reduction in the area infested.

After the second treatment, direct observation in the larger sewers (which can be entered by men), showed that rats, which had previously been numerous and observable at every point, were by then rare. Several local authorites reported shortly afterwards that the number of reports of surface rat infestations had fallen, evidently as a result of the treatment of the sewers.

Since the completion of the London scheme, nearly every large built-up area in England and Wales has been similarly treated with, as far as can be found out, similar results.

These initial treatments, designed to kill the mass of the sewer rats, have had to be followed by further treatments on a smaller scale at regular intervals. The interval recommended is 6 months. Fig. 2 illustrates the result of carrying out this recommendation in one instance (City of London). Damp sausage rusk and zinc phosphide were used in treatments 1, 3, 4 and 6; wet bread and barium carbonate in treatments 2 and 5. It is unlikely that the use of the same bait materials in successive treatments was of importance in this instance, since there is no evidence that bait shyness persists in wild populations for as long as 6 months.

Comparison of the figures for the first and second treatments, which formed part of the London scheme described above, illustrates the effect of the first treatment. The total number of infested manholes was rather more than halved. Detailed analysis of the amount of bait estimated to have been eaten indicates that the rat population was a good deal more than halved. The daily take of bait in each manhole was recorded as 'complete' ($C$), 'good' ($G = > \frac{1}{2}$), 'small' ($S = < \frac{1}{2}$), and 'no take' ($NT$); this recording has been exceptionally well supervised in this borough and can probably be relied upon to give a fairly accurate picture. It will be noticed that in the second treatment $C$ prebait takes are reduced from 49 to just over 5%, and $C + G$ poison takes are reduced from 23 to 10%. These figures, like those for the whole London scheme, indicate a great reduction in rat numbers at the manholes that initially had particularly heavy populations.

J. S. Perry (see *Bureau of Animal Population Reports*) confirmed the success of these initial treatments by sample censuses in fifty-three out of the total of 447 manholes. He concluded that over 90% of the original population had been destroyed.

After the initial treatments, there was an interval of a year, during which the infestation was largely restored. It seems likely that the restoration of infestation was mainly a consequence of breeding rather than of invasion from surface premises, since, on the whole, the surface infestations in this particular area are of *R. rattus* rather than *R. norvegicus*.

The other main feature of interest in the figures for this borough is the steady reduction in both extent and intensity of infestation as a result of 6-monthly treatments: the reduction in extent is shown by the overall figures, with their indication of reduction in the number of manholes infested; the reduction in intensity is shown most clearly by the disappearance of complete takes in the last two treatments.

This is the most complete and extensive set of records we have of the effects of sewer treatments in any locality. The results of treatment in other areas show a similar pattern in general, but there is, as might be expected, quite considerable local variation. This variation is doubtless due to the operation of many factors, including the character of the sewer systems, the initial intensity of surface infestations with *R. norvegicus* and the energy with which the extermination of rats in surface premises is carried out.

## 4. Conclusions

Even so brief a survey must make it clear that the task of rat destruction involves the most intimate collaboration between science and administration. The successes of the wartime rat campaign in England and Wales have undoubtedly been largely due to the fact that suitable relationships were developed between the organization undertaking research, the central administrative and scientific bodies, and the authorities concerned directly with rat destruction locally throughout the country. As far as rodent control in towns was concerned, the bodies were the Bureau of Animal Population at Oxford (research); the Ministry of Food Infestation Control Headquarters (administration and scientific advice and education); and the Ministry of Food divisional rodent organizations and the Health Departments of the local authorities (rodent destruction). To these must be added a fourth element in the situation: the general public, whose aid was enlisted by various means, including posters, exhibitions, films and leaflets. The aim was to convey to the public a sufficient understanding of the principles and objects of modern methods for them to play their part in reporting infestations and in agreeing to the appropriate measures being carried out on their own property.

The work of rodent control is therefore a small instance of at least partial success in the great task of making science available to the whole community.

Up to April 1945, H. R. Hewer was in charge of the scientific side of the rodent work in the Ministry of Food, and played a corresponding part in the early planning of control measures, and notably of the London sewer scheme. Miss S. M. Willmott and Mrs B. Izmidlian have helped in the analysis of records. A. G. Jenson, F.R.I.B.A., drew the figures. J. Carter, Rodent Officer to the City of London, kindly provided the data for Fig. 2. To all these, and to other members of the Ministry's staff who have contributed, the present author is much indebted.

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# REVIEWS

*Botany: Principles and Problems.* By EDMUND W. SINNOTT. 4th ed. Pp. xvii + 726, with a Frontispiece and 403 Text-figures. New York and London: McGraw-Hill Book Company, Inc. 1946. 22*s.* 6*d.*

This volume by the Professor of Botany in Yale University, first published in 1923 and now appearing in its fourth edition, is better known to American than to British students but its merits should ensure for it a warm welcome in this country. The book is accurate and more up to date than many corresponding volumes; it is clearly and interestingly written and well illustrated; and it covers the field in a logical and admirably balanced way to a standard which is approximately that of University 'Intermediate'. Each chapter concludes with a series of intriguing 'Questions for Thought and Discussion', but there are no suggestions for further reading.

A marked and welcome feature is the attention given to the economic importance of various plant structures and products and the families from which they are derived; this is not something just 'stuck on' to the end of an academic consideration but is an integral part of the author's approach to the subject. The student is also introduced to ideas on antibiotics, plant tissue culture, photoperiodism, allometric growth, soilless cultivation, viruses, the use of tracer isotopes, electron microscopy, fungal genetics, the relation of genetics to evolution, growth substances, vitamins, and other modern problems which find a rightful place in to-day's perspective. Indeed, I know of no other introductory text-book of general botany in which principles, the research attitude and current problems are interwoven so interestingly into the structure of the book or maintained so consistently before the reader.

There is a new and excellent chapter on 'Plant Distribution'; the chapter on the 'Bryophyta' has been entirely rewritten by Prof. Hempstead Castle; and in an interesting final chapter, 'Botany and the Future', the author surveys briefly some of the more active fields of botanical research and looks—rather conservatively—into the future. There is also an Appendix containing the most important Greek and Latin roots used in the construction of botanical terms. The book is attractively produced and the price is very reasonable. Many American text-books are much too American for English use but, although the present work also bears special reference to North American conditions and needs it does so to a commendably slight extent and, from any point of view, it is a first-class general introduction to plant science. In my early student days I should have revelled in a book of this character.

WILLIAM B. BRIERLEY

*Principles of Animal Biology.* By PROF. A. FRANKLIN SHULL, with the collaboration of PROF. GEORGE R. LARUE and ALEXANDER G. RUTHVEN. 6th ed. Pp. x + 425. McGraw-Hill Publications in the Zoological Series. New York and London: McGraw-Hill Book Company Inc. 1946. 20*s.* net.

Prof. Shull's book is dedicated to 'the Teachers of Zoology who have beheld their subject outgrow a pedagogical method'; it was one of the first text-books to deviate from the old method of studying biology by giving an account of structure and anatomical comparisons, and instead, making the subject a living one based on growth, function and behaviour. Its intrinsic value may be assessed from the fact that it has passed through five editions since it was first published in 1920.

Chapter 1, on the growth and scope of biology, is a particularly good introduction to the subject. Chapters 2 and 3 assume that the reader has had no grounding whatever in scientific

subjects, but it is hardly conceivable these days that pupils reading this book should need to be told that 'matter exists in a number of different forms called elements'. Subsequent chapters deal with cell division, movement, respiration and transportation. There are very good chapters on breeding behaviour, embryonic development and genetics, and an excellent feature is a chapter on the principles of taxonomy. The modern and refreshing manner in which the book deals with ecology, geographical distribution and evolution has been noted in earlier reviews and needs no further comment here except to say that marine habitats have been added to the chapter on ecology, and the historical account of zoogeography and the section on prehistoric man have been enlarged.

Although deservedly popular in the United States, in common with other biological text-books, it has not become a popular text-book in this country. This is understandable when it is realized that in the references to the literature at the end of each chapter only very few British works are quoted.

Although no radical changes have been made, this, the sixth edition, has been considerably revised. The treatment of function has been emphasized and brought up to date and this applies in particular to the section on enzymes, vitamins, and endocrines.

The book is illustrated by over 300 figures, and in this edition a very welcome improvement has been made by replacing some of the older borrowed figures by simplified diagrams. The glossary is an excellent feature but an explanation of the derivation of terms would considerably enhance its value. I. THOMAS

*A Practical Course in Agricultural Chemistry*. 2nd ed. By FRANK KNOWLES and J. ELPHIN WATKIN. Pp. viii + 216. London: Macmillan and Co., Ltd. 1947. 12*s*. 6*d*.

With the considerable advances that have been made in Agricultural Chemistry since the first edition of this book appeared in 1936, the authors have had no easy task in trying to produce an up-to-date and comprehensive practical course at a price within the reach of the student. Yet Soils, Fertilizers and Manures, Plant and Animal Biochemistry, Feeding Stuffs, Dairy Products, the Chemical Examination of Water and Insecticides and Fungicides are all dealt with in this volume, and, in the main, the practical exercises have been carefully chosen. Few references are given, but a very commendable feature is the brief preliminary discussion of the occurrence and agricultural importance of each substance to be examined. In all cases the theoretical basis of the determination is outlined and the appropriate factors for calculation given.

With so wide a field, it is inevitable that certain subjects should receive but brief treatment. Silage finds no mention at all, and while a lengthy separation of plastid pigments is described, no qualitative tests for cell-sap pigments are given. Rapid plant-tissue tests, dismissed by the authors as unsuitable for the student, could, with advantage, replace the long complete analysis of plant material introduced in the new edition. Another omission is the total neutralizing value of a liming material, a determination of particular use in assessing the quality of a waste lime. Rapid assessment of the textural type of a soil by feeling and moulding the moist soil might well have found mention as a preliminary to a complete mechanical analysis.

In bringing a book of this description up to date it is indeed unfortunate that dinitro-*o*-cresol should receive only brief mention in the *addenda*. Qualitative tests for this important compound should certainly have been given, and since there is a simple method of estimation available, this might also have been included.

Few chemistry courses for Agricultural students could spare time to undertake such lengthy estimations as the determination of soil mineral ingredients soluble in hydrochloric acid or a detailed chemical analysis of water. On the other hand, a welcome addition in the soil chapter is the introduction of a rapid method of assessing available phosphate and potash,

although the modified Morgan method, now in routine use at several soil advisory centres in the country might have been put forward in place of the Hellige-Truog method described. By using previously prepared standards a student can analyse several soils in the course of a single practical period, grading his results as high, low, etc., without the need for any special apparatus. Other valuable introductions in this chapter are the electrometric method of *p*H measurement by means of the glass electrode, brief experiments on nitrification and ammonification, determination of total exchangeable bases and exchangeable hydrogen, and the dichromate method of determining organic carbon.

The fertilizer chapter has undergone little change in the new edition but triple superphosphate, agricultural salt and sewage sludge now rightly find mention.

The methods of analysis given for D.D.T. (p. 194) and hexachloro-*cyclo*hexane (p. 195) are both subject to errors which apparently have not been appreciated by the authors. In the D.D.T. estimation the action of boiling *N* potassium hydroxide for 30 min. is known to release more than one chloride ion per molecule, thus leading to high results. Further, since D.D.T. is almost completely dehydrohalogenated in 5 min. by this reagent at room temperature, the use of the 'blank' estimation as described invites serious error. With the mixed isomers of hexachloro-*cyclo*hexane the error introduced in the blank estimation recommended might also be considerable, for this material loses hydrogen chloride rapidly to alcoholic alkali at ordinary temperatures.

The authors are to be congratulated on the clear and simple method of presentation adopted throughout this volume, and the above criticisms, it is hoped, will not weigh too heavily against its many excellent qualities, not the least of which is the useful Appendix on Reagents. The volume can be confidently recommended for the purpose for which it is intended.

R. L. WAIN